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The effect of 2.4-D acid on green and blue-green algae in unialgal and mixed cultures*

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Abstract — Low concentrations of 2.4-D usually stimulated the growth of algae. Higher concentrations inhibited or stopped the growth. Chlorococcal green algae were more sensitive than filamentous green and blue-green algae. Two or four sensitive and tolerant species were grown together in mixed cultures treated with 2.4-D. Tolerant species decreased the toxicity of the herbicide to sensitive algae. The protective effect did not appear when *Scenedesmus acutus* was used as the tolerant species.

Key words: green algae, blue-green algae, sensibility to 2.4-D.

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1. Introduction

The derivatives of 2.4-D are among the most popularly used herbicides (Taylor et al. 1972, Książek 1976): hence, growing amounts of these compounds have been noted in aquatic environments (Taylor et al. 1972). In a previous work (Bednarz 1981) the sensibility of 12 algae species to 9 pesticides, among them to the 2.4-D acid, was determined under laboratory conditions. Variable response of the algae and a wide range of toxic concentrations (from 0.15 μg—1000 mg · dm^{-3}) were found. However, the results concerning the sensibility of the algae were obtained in unialgal cultures under laboratory conditions (Maloney 1958, Arvik et al. 1971, Kruglov 1975, Bednarz 1981) and they could have a relation to outdoor conditions where unialgal populations are extremely rare. Apart from the toxic action, the effect of pesticides on algae communities can be manifested both by enhanced

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or reduced action of one group of organisms on the other. Therefore, besides the detailed investigation on the effect of 2.4-D on the kinetics of growth of 12 algae species in unialgal cultures, differences in the sensitivity of species previously classified as sensitive or tolerant of 2.4-D, were analysed under conditions of mixed cultures.

The investigation was carried out in the years 1975—1979. It was begun in the Institute of Zootechnics at Zator and continued in the Laboratory of Water Biology of the Polish Academy of Sciences in Cracow.

2. Material and method

The experimental materials included 12 algologically pure cultures of algae. Among them were 9 species of *Chlorophyceae: Chlorella pyrenoidosa* Chick., strain No 366, *C. mucosa* Korschik., strain No 594, *Ankistrodesmus minutissimus* Korschik., strain No 1193, *Chlorococcum* sp., No 564, *Scenedesmus acutus* Meyen, No 1608, *S. quadricauda* (Turp.) Bréb., No 1097, *Dictyosphaerium pulchellum* sp., No 1619, and three *Cyanophyceae* species: *Anabaena variabilis* Kütz., No 1618, *Spirulina platensis* (Gom.) Geitl., No 1620, and *Oscillatoria* sp., No 1621. The cultures of algae were obtained from the collection of the Institute of Zootechnics at Zator (Bednarz, Nowak 1971). A chemically pure preparation of 2.4-D was obtained from the Pedagogical Institute at Köthen (GDR).

The cultures were conducted up to the stationary phase of growth, i.e., during 14 days, in the liquid L_4m medium (Jankowski 1964) or in the Zarrouck medium (1966). The experimental conditions and the method of recording the growth were given previously (Bednarz 1981).

Mixed cultures were composed of 2 or 4 species grown in one dish. The following combinations of species were used: *Chlorella pyrenoidosa* + *Hormidium flaccidum*, *Dictyosphaerium pulchellum* + *Hormidium flaccidium*, *Scenedesmus quadricauda* + *Hormidium flaccidum*, *Chlorella pyrenoidosa* + *Scenedesmus acutus*, and *Dictyosphaerium pulchellum* + *Scenedesmus acutus*. The first component of each combination was sensitive to 2.4-D, and the other was tolerant of it. In the cultures composed of 4 species the following combinations were applied: *Scenedesmus quadricauda* + *Chlorella pyrenoidosa* + *Dictyosphaerium pulchellum* + *Scenedesmus acutus*, with three sensitive and one tolerant species, and *Chlorella pyrenoidosa* + *Hormidium flaccidum* + *Stichococcus* sp. + *Anabaena variabilis*, with one sensitive and three tolerant species.

The initial density of each component of the mixed cultures was 200 000 cells per 1 cm^3, except for *Anabaena variabilis* with an initial density of 1000 filaments per 1 cm^3. A culture of *Chlorella pyrenoidosa*

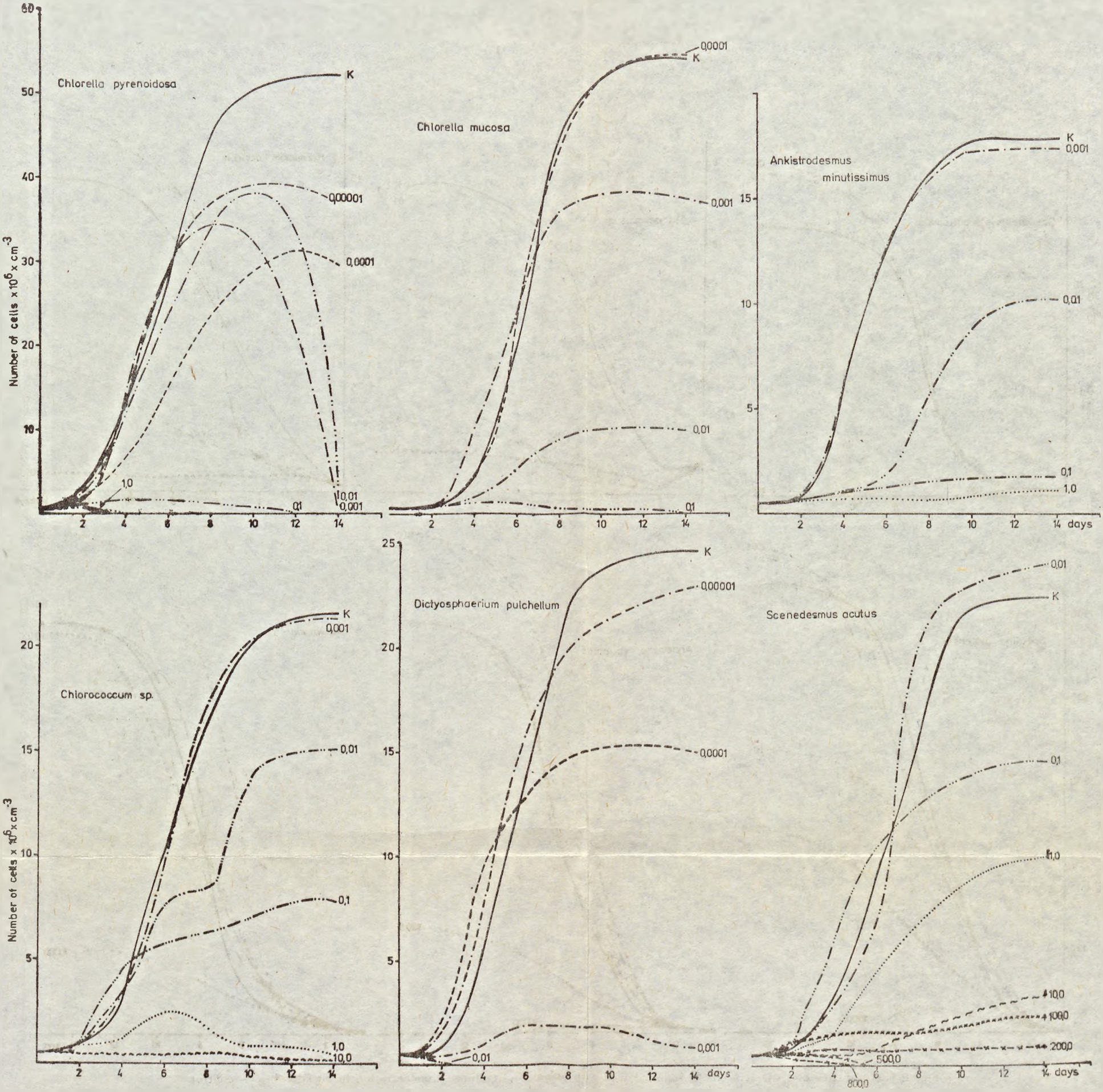

Fig. 1. The effect of 2.4-D acid on the kinetics of growth of unialgal cultures. The concentration in mg · dm^{-3}. K — control

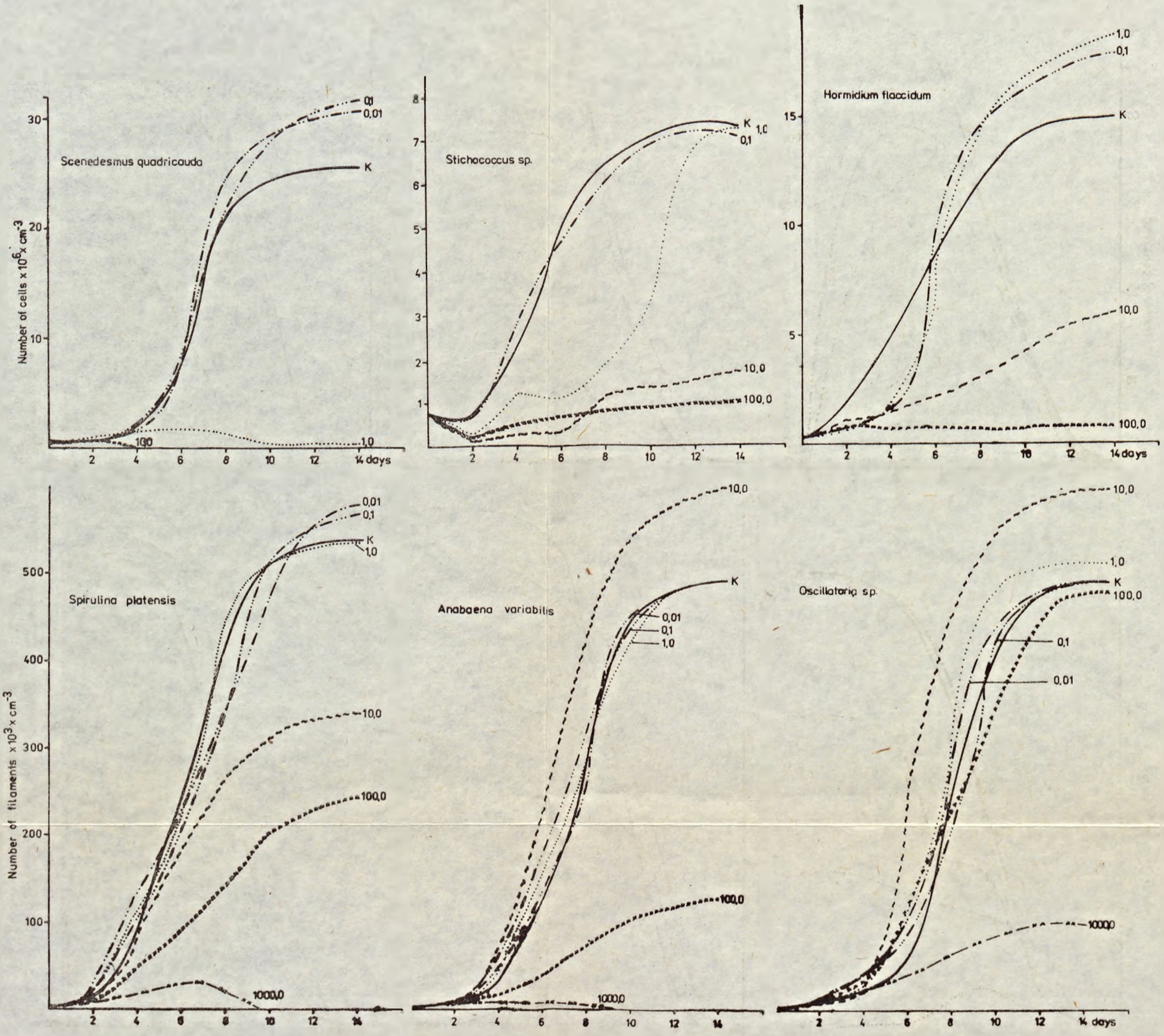

Fig. 2. The influence of 2.4-D acid on the kinetics of growth of unialgal cultures. Concentration in mg · dm^{-3}. K — control

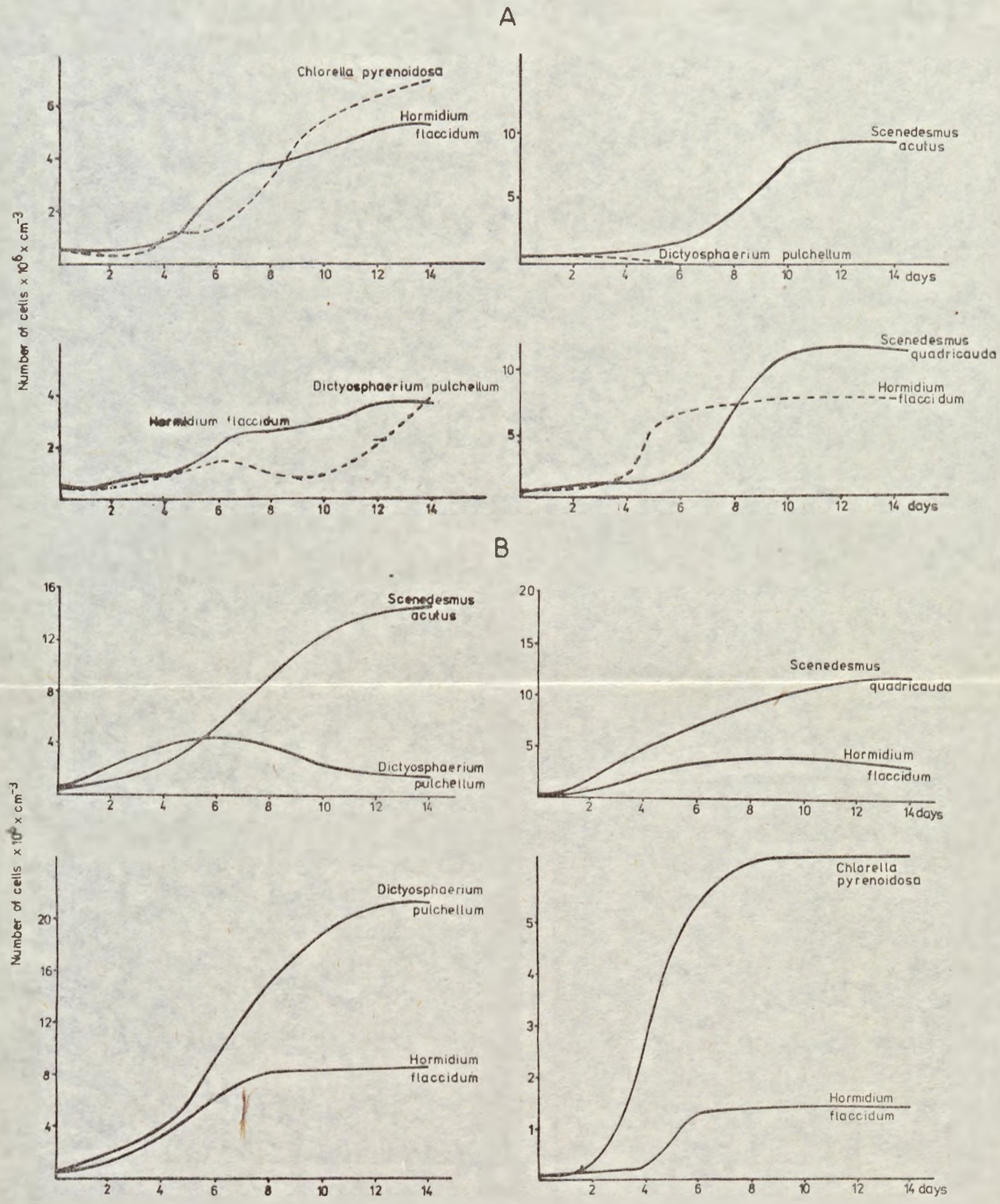

Fig. 3. The growth of mixed cultures composed of 2 species: A — treated with 1 mg · dm^{-3} of 2.4-D; B — control

+ *Hormidium flaccidum* with the inoculum density of *Chlorella* 20 times greater than the number of *Hormidium* was also conducted.

At the moment of inoculation, unialgal cultures were treated with 2.4-D at a concentration of 0.0001—100 mg · dm^{-3} with a tenfold gradation of concentrations. For some tolerant species the concentration was increased to 1 g · dm^{-3} while the most sensitive were treated with a dose of 0.00001 mg · dm^{-3}.

Mixed cultures were treated with 2.4-D at concentrations of 1 and 10 mg · dm^{-3}. The applied doses were toxic for sensitive species, being tolerated by resistant algae (B e d n a r z 1981).

The cultures were conducted in three replications. Untreated cultures of algae were established as the control.

3. Results

3.1. The effect of 2,4-D on the growth of unialgal cultures

The herbicide brought about numerous modifications in the growth of the investigated species. The extension or shortening of the lag phase, the stimulation within the exponential phase, the increased or decreased rate of cell divisions in the phase of linear growth, the appearance of transitory periods of inhibition in this phase, and the earlier or later appearance of the stationary phase were observed in the cultures (figs 1—2). In a few cases the influence of the pesticide was limited to the elimination of some phases of growth, e.g., when the linear phase was reached, the rate of cell division grew, being similar to that in the control. Afterwards, without the stationary phase, a rapid dying-off of the algae occurred. Sometimes, an opposite effect was observed, with a slow but constant growth of algae from the first day of the experiment. In a few species the action of 2.4-D was manifested either by growth inhibition at the inoculum level or by the stimulation of growth during whole course of the culture development (figs 1—2).

3.2. The effect of 2.4-D on the growth of mixed cultures

In the cultures composed of two species the dose of 1 mg · dm^{-3} of 2.4-D did not stop the growth of sensitive algae (fig. 3A). The toxic action of the herbicide was only found in the combination of *Dictyosphaerium pulchellum* + *Scenedesmus acutus*, where the growth inhibition and the dying-off of the sensitive species during 6 days, occurred (fig. 3A).

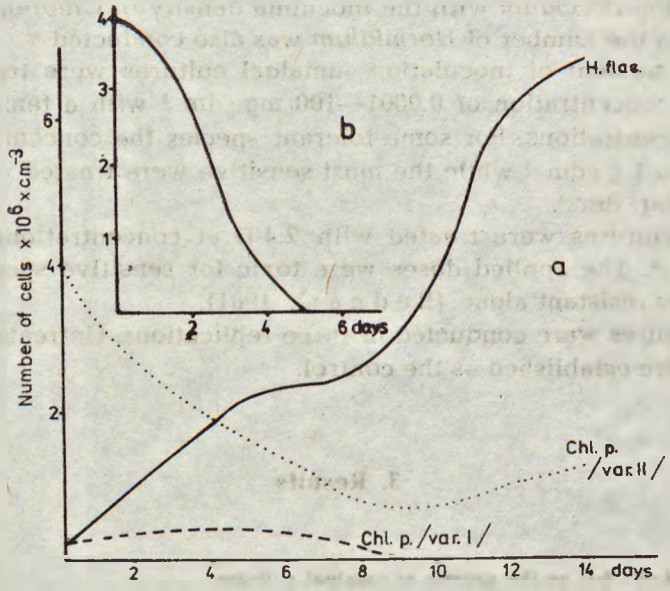

Fig. 4. The growth of algae cultures treated with 10 mg · dm^{-3} of 2.4-D: a — mixed culture of *Chlorella pyrenoidosa* and *Hormidium flaccidum*. Variant I — equal density of *Ch. pyrenoidosa* and *H. flaccidum*; Variant II — the 20-times greater density of *Ch. pyrenoidosa* inoculum; b — unialgal culture of *Chlorella pyrenoidosa* with high initial density of inoculum

In a mixed culture of *Chlorella pyrenoidosa* + *Hormidium flaccidum* treated with a dose of 10 mg · dm^{-3}, the pesticide inhibited the growth of the sensitive species *Chlorella pyrenoidosa* and killed it after 9 days (fig. 4a, variant I). The application of *Chlorella pyrenoidosa* inoculum with 20-times greater initial density, brought about a decline in the number in cells. The decline occurred during 9 days, then a slow increase began and was carried on until the and of the culture (fig. 4, variant II). It should be mentioned that in unialgal cultures the doss of 10 mg · dm^{-3} killed *Chlorella pyrenoidosa* of the same initial density, during 6 days (fig. 4b).

In cultures composed of one sensitive and three tolerant species treated with 10 mg · dm^{3-}, the growth of *Chlorella pyrenoidosa* was better (fig. 6a) than in parallel untreated cultures (fig. 6b). However, it was observed that the lag phase of growth of *Chlorella pyrenoidosa* was extended, with the further development of phases unchanged (fig. 6).

Mixed cultures composed of three sensitive and one tolerant species, treated with the pesticide at the dose of 10 mg · dm^{-3} were killed after 3 days. The dose of 1 mg · dm^{-3} was toxic to *Chlorella pyrenoidosa* and *Dictyosphaerium pulchellum*. It inhibited the growth of *Scenedesmus*

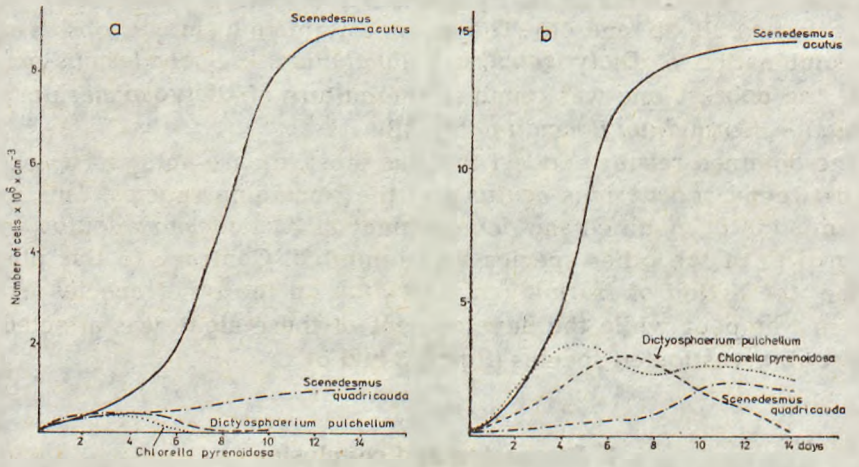

Fig. 5. The growth of mixed cultures composed of 4 species: a — treated with 1 mg · mg^{-3} of 2.4-D; b — control

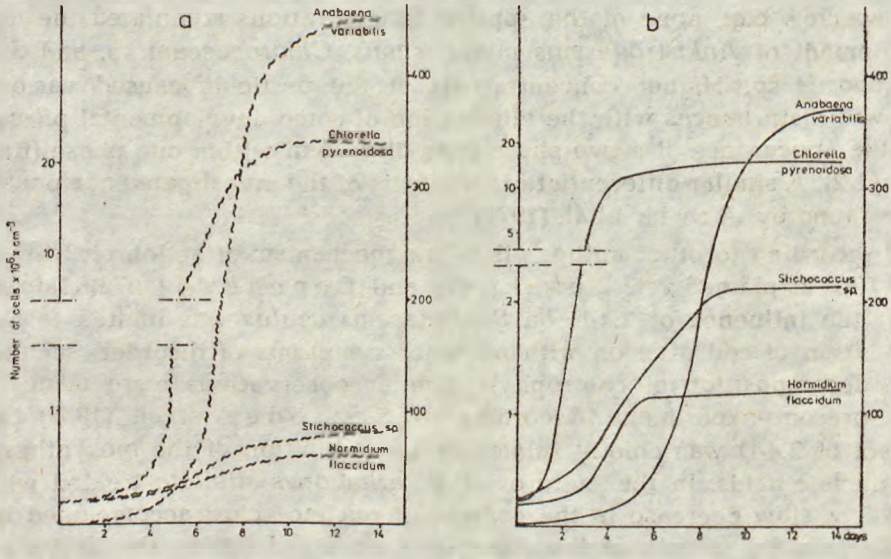

Fig. 6. The growth of mixed cultures composed of 4 species: a — treated with 10 mg · dm^{-3} of 2.4-D; b — control

quadricauda but did not affect the development of *Scenedesmus acutus* (fig. 5a).

Differences were observed in the growth of the investigated species in mixed and unialgal cultures both treated and untreated with 2.4-D (fig 1—6). The interaction between the species was manifested by the

inhibition of growth which was particularly intense in the mixed control cultures (figs 3B, 5b, and 6b). The strongest antagonism was observed in the combination of *Dictyosphaerium pulchellum* + *Scenedesmus acutus* while the poorest one was found in the culture of *Dictyosphaerium pulchellum* + *Hormidium flaccidum* (fig. 3B).

The obtained results suggest that the most intense antagonism occurred between *Scenedesmus acutus* and the remaining species. This antagonism slightly modified the development of *Scenedesmus acutus* while the growth of the other species was inhibited. Contrary to this phenomenon, the action of *Hormidium flaccidum* on the other species in the culture was poor while the development of these algae was affected by almost all investigated species (figs 2, 3 and 6).

4. Discussion and conclusions

Different effects of 2.4-D on the kinetics of growth of algae cultures were observed at all investigated ranges of concentrations. Usually, low concentrations stimulated the growth, but this effect was not found with all species. E.g., none of the applied concentrations stimulated the development of *Ankistrodesmus minutissimus*, *Chlorococcum* sp. and *Stichococcus* sp. Higher concentrations of the pesticide caused various growth disturbances with the elimination of some developmental phases or the appearence of a two-phase type of growth within one phase (figs 1 and 2). A similar differentiation of effects of the investigated compound was found by Arvik et al. (1971).

According to other author's data the mechanism of action of 2.4-D is not fully explained yet. Dushkova and Dencheva (1973) claimed that the influence of 2.4-D on *Scenedesmus acutus* was limited to the inhibition of cell division with no other symptoms of disorder, such as the decomposition of chlorophyll. Similar observations were made in the present experiment. According to Schröder et al. (1967) the effect of 2.4-D was chiefly related to the inhibition of the biosynthesis of nucleic acids. In the cultures of *Poteriomonas stipitata* treated with 2.4-D, a slow decrease in the content of nucleic acids, accompanied by a fairly good growth of the algae, was observed during the first nine days. It was followed by a drop in the amount of nucleic acids and the death of the algae after this period. Also in the present experiment, the cultures of *Chlorella pyrenoidosa* were similary killed after nine days by low concentrations of the herbicide (fig. 1).

The toxicity of 2.4-D was more pronounced with regard to small chlorococcous green algae than to filamentous green and blue-green algae. The greatest sensibility to this herbicide was manifested by the species of the genera *Chlorella* and *Dictyosphaerium* with acutely reac-

ted to the dose of 0.1 mg · dm^{-3}. Similar data on the toxicity of this compound were given by Arvik et al. (1971). Contrary to Arvik's findings, no toxic effect of 2.4-D for *Chlorella* either at the concentration of 50 mg · dm^{-3} (Fletcher, Kirkwood 1970) or at higher concentrations (Bertagnolli, Nadakavukaren 1974) were found by other authors. Valentine and Bingham (1974) reported that the concentration of 200 mg · dm^{-3} was not toxic for *Scenedesmus quadricauda;* the present author, however, observed this toxicity already at the concentration of 1.0 mg · dm^{-3}. Considerable toxicity of 2.4-D to *Scenedesmus acutus,* with the dose of 50 mg · dm^{-3} completely stopping its development, was observed by Dushkova and Dencheva (1973). In the present study the respective concentration amounted to 200 mg · dm^{-3}.

The discrepancies between the data quoted by various authors on the toxicity of 2.4-D to algae cultures, were probably caused by different methods used in the experiments. Most observations on the effects of 2.4-D on the growth of algae were conducted during short periods, this making it impossible to investigate the chronic sensibility of algae to this pesticide.

In mixed cultures the algae species mutually affect their growth. Usually, the development of the components of mixed cultures is less intense than the growth of these species in unialgal cultures (Jørgensen 1959, Krzywicka, Krupa 1975, Mur et al. 1978, Bednarz, Cierniak 1979). Modifications in the growth of mixed cultures are caused both by the action of extracellular substances secreted by the algae to the medium (Jørgensen 1956, Fogg 1962, Matusiak et al. 1965) and by the competition for nutrient compounds. The results of Bednarz and Cierniak (1979) showed that the filtrates obtained from the culture of *Hormidium flaccidum* and *Anabaena variabilis* rather stimulated the growth of *Chlorella pyrenoidosa, Dictyosphaerium pulchellum* and *Scenedesmus quadricauda,* while in parallelly conducted mixed cultures of these species a slight growth inhibition was observed. The present results also suggest a slight antagonism between *Hormidium flaccidum* and *Chlorella pyrenoidosa, Dictyosphaerium pulchellum* and *Scenedesmus quadricauda.* In the mixed cultures of these species treated with 2.4-D a considerable reduction of toxicity of the herbicide to the sensitive species was brought about by the protective action of tolerant species. The different response noted in the mixed culture with *Scenedesmus acutus* as the tolerant species, could be explained by the joint action of the herbicide and the extracellular secretion of the algae.

Bulding et al. (1970) investigated the effect of 2.4-D on the mixed cultures of algae. He found the dominance of *Pediastrum* in the combination of *Aphanizomenon flos-aque* + *Pediastrum duplex,* and of *Phormidium* in the combination of *Phormidium* sp. + *Chlorococcus interme-*

dium, while in the control cultures the other species prevailed. According to this author the growth of *Aphanizomenon* and *Chlorococcum* was inhibited by the influence of the pesticide, this offering better trophic conditions for the other component which could, therefore, reach quantitative dominance in the culture. Similarly, Mosser et al. (1972) observed changes in the composition of the mixed cultures of *Dunaliella tertiolecta* + *Thalasiosira pseudonana* treated with DDT. In control cultures *Thalasiosira* predominated but the domination changed under the influence of the pesticide. Additionally, in the mixed culture of these species, the sensibility of *Thalasiosira* to DDT was increased. The concentrations of this compounds which had no effect on unialgal cultures of *Thalasiosira*, proved toxic to this species in the mixed culture. The author claimed that this could be explained by the food competition of the investigated species.

The results obtained in the present investigation suggest that both the observed changes in the domination of species in the mixed cultures treated with the pesticide, and the increased sensibility of some species cannot be explained by food competition. They are rather caused by the action of the extracellular secretion of algae. In the cultures conducted on synthetic mineral media which provide conditions for the dynamic growth of the algae (Jankowski 1964, Mayers 1953, Tamiya et al. 1953) the food composition were not distinctly significant. However, extracellular secretion can be inhibited by the action of the pesticide while low concentrations of the secreted substances can stimulate the growth of algae (Pratt, Fong 1940, Fogg 1962, Lefèvre 1964). It is also possible that the joint action of pesticides and extracellular secretions can increase the sensibility of algae to the pesticides.

5. Polish summary

Wpływ kwasu 2,4-D na wzrost jednogatunkowych i mieszanych kultur glonów

Określono wpływ kwasu 2,4-D na kinetykę wzrostu gatunków zielenic: *Chlorella pyrenoidosa*, *C. mucosa*, *Chlorococcum* sp., *Ankistrodesmus minutissimus*, *Dictyosphaerium pulchellum*, *Scenedesmus acutus*, *S. qucadricauda*, *Hormidium flaccidum*, *Stichococcus* sp. i 3 gatunki sinic: *Anabaena variabilis*, *Spirulina platensis* i *Oscillatoria* sp. Stwierdzono większą toksyczność preparatu dla drobnych chlorokokkalnych zielenic, niż dla sinic i nitkowatych zielenic (ryc. 1, 2).

Obserwacje prowadzone na kulturach mieszanych wykazały, że gatunki odporne na działanie kwasu 2,4-D wpływały na znaczne obniżenie toksyczności tego preparatu dla gatunków wrażliwych. W czterogatunkowych hodowlach mieszanych (*Chlorella pyrenoidosa* — gatunek wrażliwy + *Hormidium flaccidum* + *Stichococcus* sp. + *Anabaena variabilis* — gatunki odporne) uzyskano lepszy niż w kombinacji kontrolnej, bez

pestycydu, wzrost gatunku wrażliwego, mimo obecności 10 mg·dh^{-3} kwasu 2,4-D, w ilości wielokrotnie przewyższającej toksyczną dawkę dla gatunku wrażliwego (ryc. 1,6a). W dwugatunkowych hodowlach, złożonych z gatunku wrażliwego i odpornego na kwas 2,4-D, poddanych działaniu 1 mg·dm^{-3} obserwowano wzrost gatunków wrażliwych (ryc. 3). Większe stężenie było jednak toksyczne (ryc. 4). Przy zastosowaniu 20 razy większego zaszczepu *Chlorella pyrenoidosa* w hodowli z *Hormidium flaccidum* uzyskano słaby wzrost glonu *Chlorella*, mimo obecności 10 mg·dm^{-3} kwasu 2,4-D (ryc. 4).

Efekt ochronny dla gatunków wrażliwych nie wystąpił w kombinacjach zawierających w swym składzie jako gatunek odporny *Scenedesmus acutus* (ryc. 3, 5).

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