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Radiosensitivity of the moss *Drepanocladus aduncus* (Hedw.) Mnkm.

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Abstract

Radiosensitivity was determined in isolated fragments of *Drepanocladus aduncus* gametophytes cultured *in vitro*, on the basis of the growth reaction to acute gamma Co-60 radiation and postirradiation survival of the plants. A high resistivity of *D. aduncus* to this radiation was noted. At 12°C a 100 per cent LD was 120 kR and at 22°C it was 160 kR. The nuclear index of radiosensitivity (ICV — interphase chromosome volume) for various gametophyte cells has a value from 1.54 to 9.00 μm^3. *Drepanocladus aduncus* plants exhibit postradiation developmental anomalies. In natural conditions they are characterised by an enhanced beta and gamma radiation activity. The plants contain Sr-90, Cs-137, much calcium, beryllium and lithium.

INTRODUCTION

Bryophytes differ from other plant groups by their relatively high natural radiation (Grodziński, 1965) and are capable of accumulating radioisotopes (Maslov et al., 1970). For this reason they are more and more frequently utilised in bioindication of radioactive contamination of the environment. A timely task of radioecology is gaining knowledge on the radiosensitivity of wild plants (Aleksakhin, 1968). The radiosensitivity of bryophytes is so far but little known and very necessary for the further development of the radioecology of these plants (Sarosiek, 1972).

The choice of the species *Drepanocladus aduncus* as object of the present investigations is not accidental. Kulikov (1971) demonstrated that the related species *Drepanocladus sendtneri* (Schimp.) Warnst. accumulates under natural and experimental conditions the same amount of Sr-90, whereas under natural conditions it cumulates three times more Cs-137 than in experimental conditions. Moreover, it is characterised by a high accumulation of these radioisotopes as compared with other macrohydrophytes. It could be expected that *Drepanocladus aduncus* will possess similar ecological properties. According to Boros and Iarai-Komlodi (1975) it is a circumboreal, almost cosmopolitic species. In Poland it is a common plant (Szafran, 1957). It is a helophyte, often hydatophyte, heliophilous, terri-turfophilous, indifferent, rather basiphilous, even halophilous. In moors of most different kinds, on damp habitats. Thus it may fulfill the criteria for plant bioindicators of radioactive contamination of the environment, and similarly as *Drepanocladus uncinatus* (Hedw.) Warnst. of chemical air pollution (Kannukene, Tamm, 1976).

Determination of the radiosensitivity of plants consists in establishing the specific developmental reaction of plants to irradiation and their survival after definite exposure doses (Sparrow, 1962). This required planned experiments with isolated fragments of *Drepanocladus aduncus* plants *in vitro* cultures. The investigations were carried out in the Department of Ecology and Nature Protection of the Institute of Botany, Wrocław University in the period 1975-1977.

METHODS

A) PLANTS

The experiments were performed on plants from a natural *Drepanocladus aduncus* population growing at the bottom of pond in Szczodre near Wrocław. This population is a component of reedswamp plant community. The plants were collected in late autumn, each time several days before starting the experiments. The apical segments 2 cm long of the main gametophyte shoots were isolated for the first preliminary experiment, and in the following ones 1 cm segments were used. Gametophyte branches of equal length were selected, degree of branching and vigorusness. After isolation they preserve their elongation growth ability and exhibit a high regeneration potential.

B) EXPERIMENTS

The isolated fragments of gametophyte branches were cultured *in vitro* in nonsterile tap water. Preliminary experiment 1, for checking the regeneration ability, the elongation rate and establishing the possibly optimal culture conditions. In this experiment temperature and light were varied. The cultures were kept in a glasshouse in combinations at 22° and 12°C and were kept in both glasshouses under full daylight and light restricted to 40 per cent (shaded cultures). The culture period lasted from December 6, 1975 to February 6, 1976. Five replications were made, each including 5 fragments of gametophyte in cristallisers. From the beginning of the culture the length increment of the gametophyte was measured at 10-day intervals and the length of its I and II order branchings, and the branchings were counted. The increment of the gametophyte branches at various temperatures and illuminations is shown in Fig. 1.

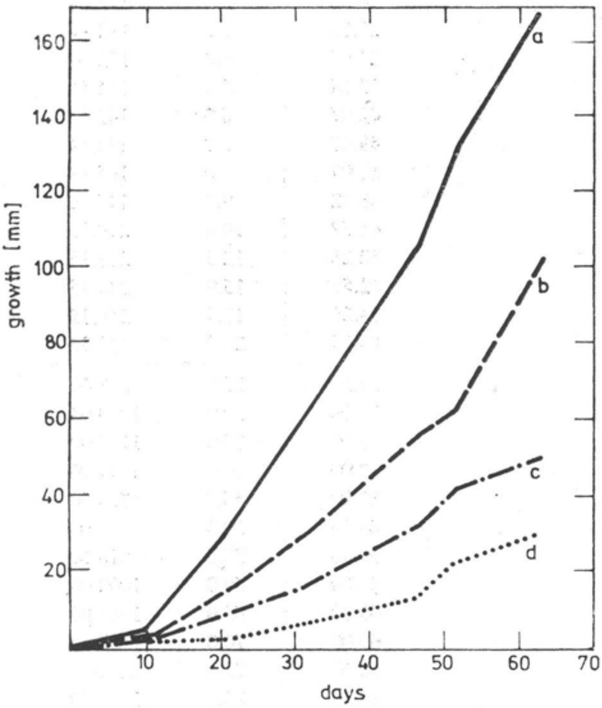

Fig. 1. Growth of main shoot of *Drepanocladus aduncus*
a — at 22°C under full light, b — at 22°C under limited light, c — at 12°C under full light, d — at 12°C under limited light

Subsequently 3 successive experiments (2, 3 and 4) were carried out. The plants were irradiated from a Co-60 source in the Laboratory of Radiobiology of the Institute of Plant Breeding and Acclimatisation in Radzików near Warsaw. In each experiment the plants were exposed to radiation on the second day after isolation of the gametophyte branches. The doses, their strength, the time of exposure and the distance from the radiation source are given in Table 1. The irradiated plants were kept in the glasshouse at 12°C and 22°C under full daylight. The temperature was differentiated according to the results of experiments and on the basis of the fact that bryophytes kept at lower temperatures have smaller cell nuclei, therefore they may exhibit varying radiosensitivity (S a r o s i e k, 1972).

Table 1

Data concerning irradiation conditions of *Drepanocladus aduncus* from Co-60 source

Experiment No	Time of exposure (hours, min)	Exposure in kR	Dose-rate R/h
2	7.01	1.0	142.44
	14.02	2.0	142.44
	21.03	3.0	142.44
	28.05	4.0	142.44
	35.06	5.0	142.44
	42.07	6.0	142.44
	49.08	7.0	142.44
	43.09	8.0	185.38
	48.32	9.0	185.38
	41.57	10.0	238.35
	52.26	12.5	238.35
	62.56	15.0	238.35
	73.25	17.5	238.35
	83.55	20.0	238.35
3	30.00	25.0	833.00
	30.00	30.0	1000.00
	30.00	35.0	1166.00
	30.00	40.0	1133.00
	60.00	45.0	750.00
	60.00	50.0	833.00
	60.00	55.0	916.00
	60.00	60.0	1000.00
	60.00	65.0	1083.00
	60.00	70.0	1167.00
	60.00	75.0	1250.00
	60.00	80.0	1333.00
4	49.40	60.0	1208.00
	48.41	70.0	1438.00
	45.16	80.0	1767.00
	50.56	90.0	1767.00
	45.04	100.0	2219.00
	47.19	105.0	2219.00
	49.34	110.0	2219.00
	51.49	115.0	2219.00
	42.20	120.0	2835.00
	44.05	125.0	2835.00
	45.51	130.0	2835.00
	47.37	135.0	2835.00
	49.23	140.0	2835.00
	51.09	145.0	2835.00
	41.29	150.0	3616.00
	42.52	155.0	3616.00
	44.15	160.0	3616.00

In all experiments the same biometric measurements were made. In experiments 2, 3 and 4 three replications were done in each combination (3 crystallisers and in each 3 plants). In experiments 3 and 4 the experiment was shortened as compared with experiment 2, and measurements and observations were performed at 7-day intervals.

C) PLANT AND WATER RADIOACTIVITY MEASUREMENTS

Radiometric measurements of the plants and water were done in the Laboratory of Radioecology of the Centre for Environment Protection in Katowice by the standardized methods of the International Atomic Energy Agency (IAEA). The total alpha, beta and gamma activity was measured by what is called the "thin-layer" method after Truthin and Shashkin (1975).

D) STATISTICAL MATHEMATICAL ANALYSIS

Analysis of variance was applied to the empirical data from factorial orthogonal experiments (with equal number of replications in each combination). The significance of differences was established by Snedecor's F test at probability level 0.05 (Oktaba, 1966). Moreover, linear correlations of two variables (increment of gametophyte and number of lateral branchings at various radiation doses) were calculated. The significance of the correlation was established by Snedecor's F test (Volk, 1965).

RESULTS

Statistical-mathematical analysis revealed significant differences between the values of the biometric traits examined in *Drepanocladus aduncus* when various radiation doses were applied at various times at temperatures of 22° and 12°C.

Within the radiation dosage range 1-20 kR (exp. 2, Jan. 16 — March 29, 1976) a stimulating influence of irradiation on growth of the main shoots of *Drepanocladus aduncus* was observed as early as 10 days after exposure. This stimulating effect becomes manifest at all dates at 22°C (Fig. 2a), whereas at 12°C this effect appears only within the range of lower doses (2, 4 and 7 kR). Higher doses (5-15 kR) stimulate but slightly at this temperature the growth of the main shoots and the highest doses (15-20 kR) markedly restrict growth (Fig. 2b).

At 22°C at the first examination stimulation of lateral branchings formation of I and II order by doses of 2-8 kR is observed with the exception of 4 kR, whereas the number of lateral branchings was

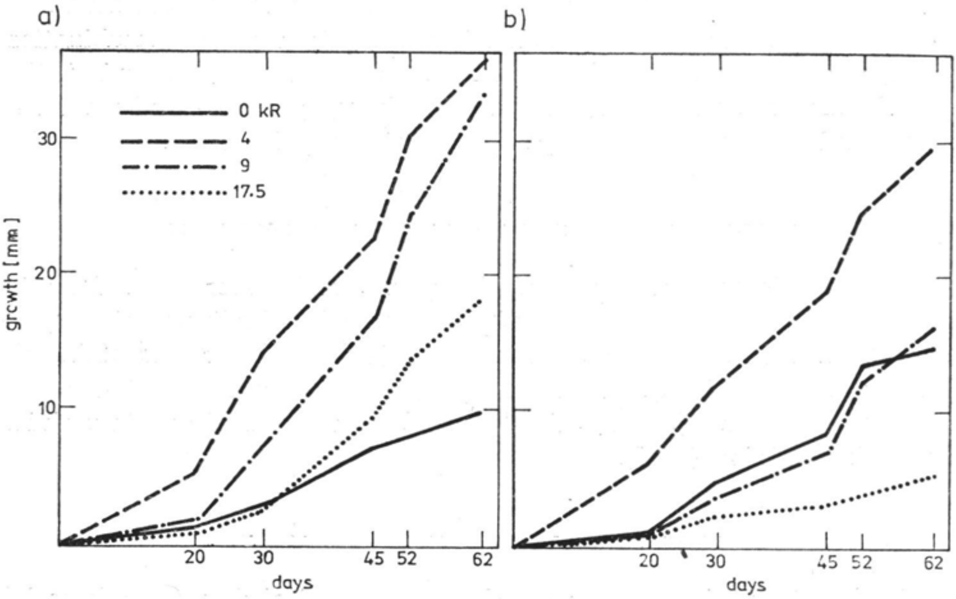

Fig. 2. Growth of *Drepanocladus aduncus* main shoot after lower radiation doses a — at 22°C, b — at 12°C

restricted at higher doses (9-20 kR, Fig. 3a). At 12°C at first the inductive effect of radiation on side branchings formation is not noticeable. Beginning with the 3rd date all doses stimulate growth of lateral shoots (Fig. 3b).

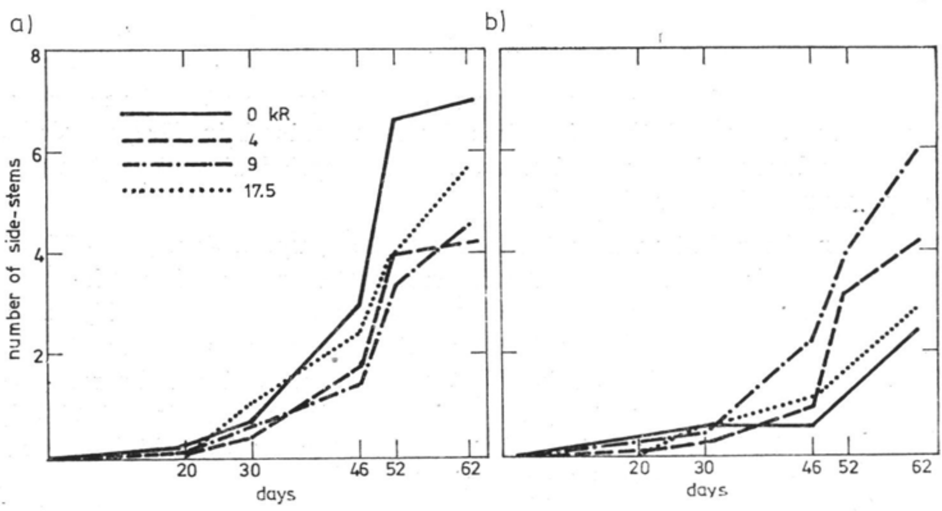

Fig. 3. Induction of side branchings formation by *Drepanocladus aduncus* after lower radiation doses
a — at 22°C, b — at 12°C

At all dates at 22°C elongation growth of side branchings of order I and II is stimulated by doses of 2-8 kR (with the exception of 4 kR), but higher doses of 9-20 kR limit growth (Fig. 4a). At 12°C a distinct stimulation of growth of the side branchings is observed (Fig. 4b).

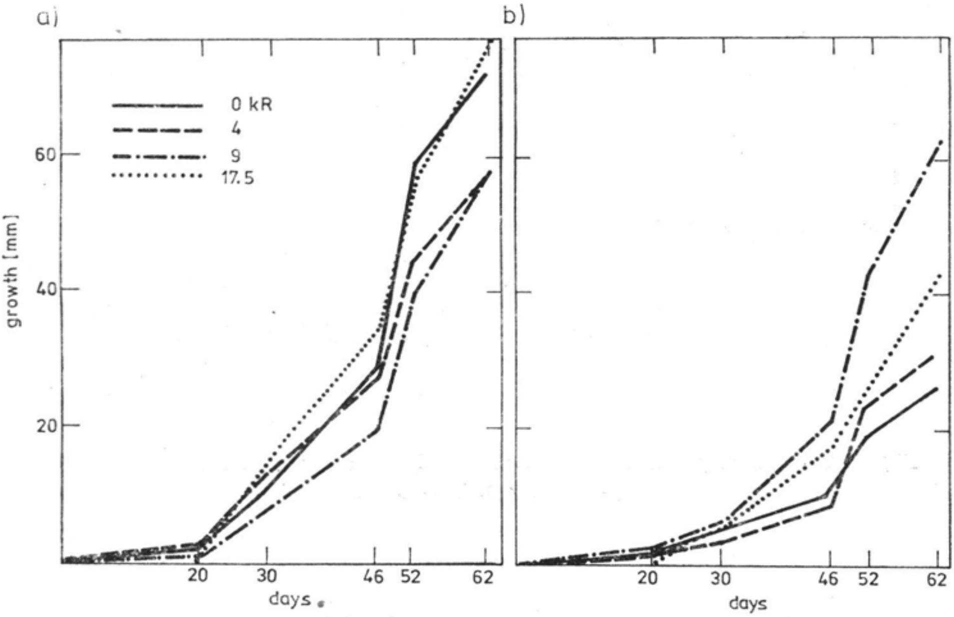

Fig. 4. Growth of side branchings of *Drepanocladus aduncus* after lower radiation doses
a — at 22°C, b — at 12°C

In experiment 3 (Nov. 26, 1976 — Jan. 13, 1977) it appeared that doses of 25-80 kR limit main shoot growth independently of the temperature in *Drepanocladus aduncus*. At 22°C the growth was inhibited stronger than at 12°C. Doses above 70 kR have a restricting effect at both temperatures, limitation of growth being noticeable only beginning with the 4th date (Fig. 5a, b).

At 22°C radiation within the whole range of doses stimulates induction of lateral branchings of order I and II and their growth at all dates. At 12°C the same doses stimulate also induction and growth of the same branchings with the exception of the highest dose of 80 kR (Fig. 6a, b). It should be metioned that the proportion of II order branchings is small, and they do not form on not irradiated plants. At 22°C the optimum of induction and growth of II order branchings is observed at a dose of 55 kR. Higher doses limit growth of II order branchings and their number. The effect is similar at 12°C, however, the branchings appear at later dates and their number and rate of growth are lower.

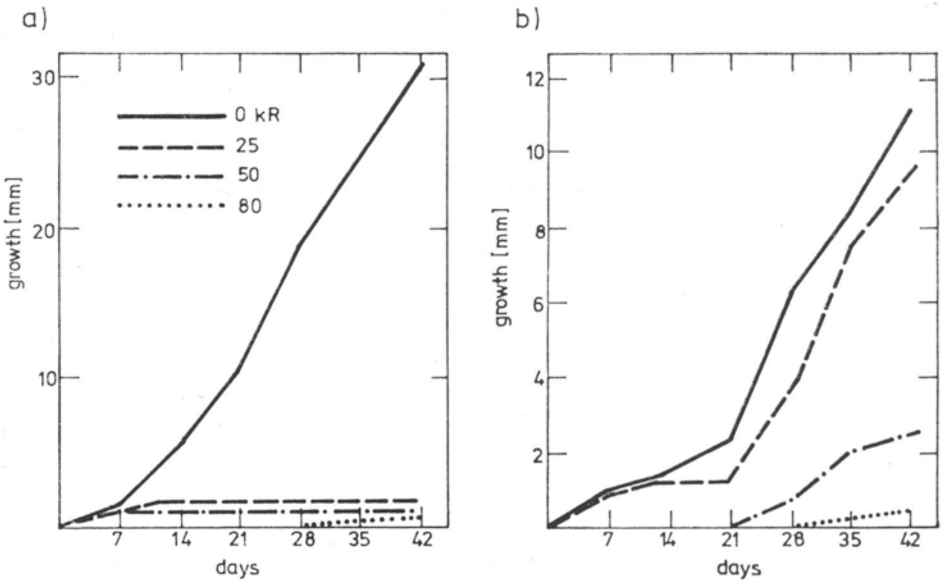

Fig. 5. Growth of main shoot of *Drepanocladus* after higher radiation doses a — at 22°C, b — at 12°C

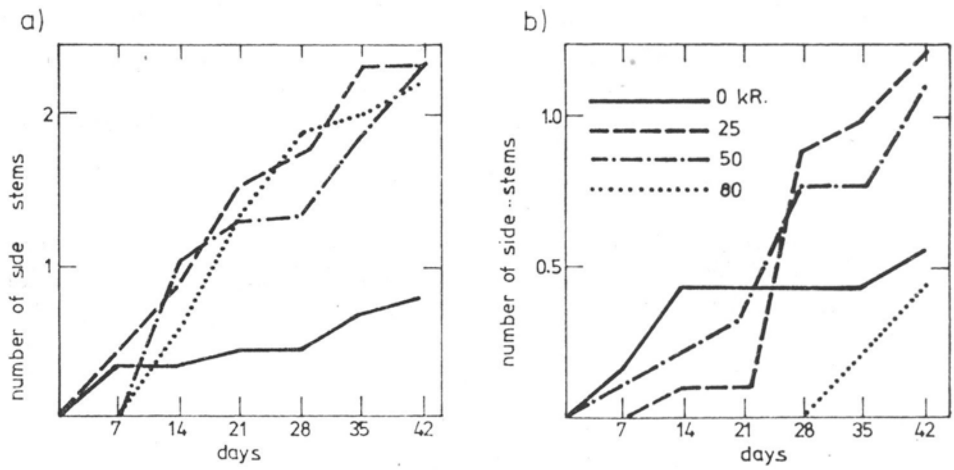

Fig. 6. Induction of side branchings formation in *Drepanocladus aduncus* after higher radiation doses
a — at 22°C, b — at 12°C

In experiment 4 (Oct. 26 — Dec. 14, 1977) the elongation growth of the main shoots under doses of 60, 70 and 80 kR is similar as in experiment 3, both at 22° and 12°C. At 12°C doses higher than 80 kR completely inhibited elongation growth of the main shoots, whereas at 22°C doses above 100 kR gave the same effect. Induction of side branchings of I and II order and their growth were still observed at doses of 155 kR at 22°C, whereas at 12°C induction of side branchings and their growth continue up to 115 kR. With increasing doses from 110 to 155 kR, at 22°C induction ceases and growth is limited (Fig. 7a, b). In this range of radiation doses growth of order I branchings is similar.

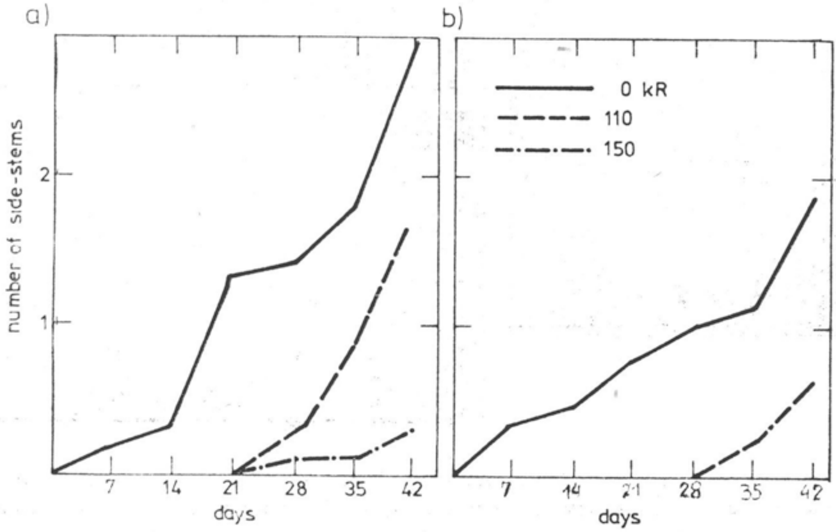

Fig. 7. Induction of side branchings formation by *Drepanocladus aduncus* at highest radiation doses
a — at 22°C, b — at 12°C

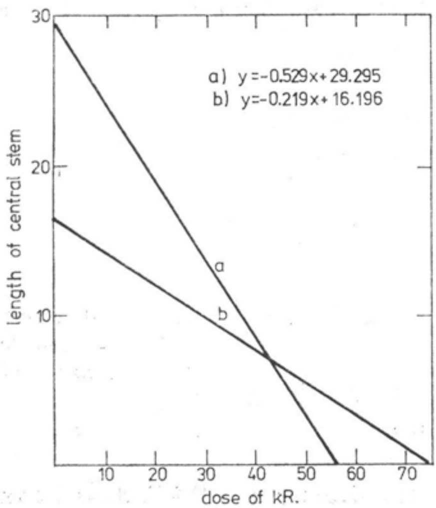

Fig. 8. Relation between main shoot length of *Drepanocladus aduncus* (y) and radiation (x)
a — at 22°C, b — at 12°C

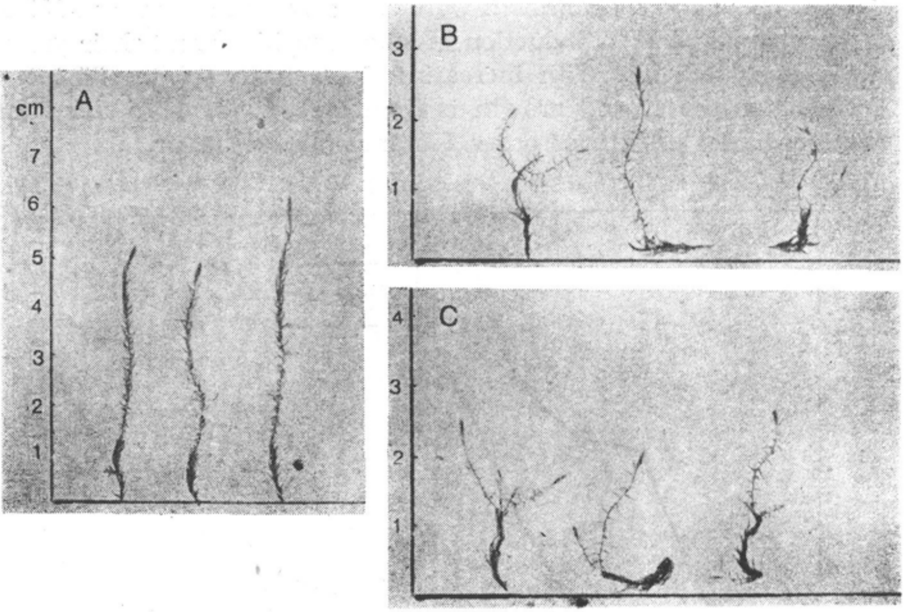

Photo. 1. Growth of isolated fragment of *Drepanocladus aduncus* shoot at 12°C after various radiation doses: a — not irradiated plants (control), b — irradiated plants with 60 kR, c — irradiated plants with 100 kR

Induction of II order branchings is observed at 22°C still with a dose of 115 kR, but at 12°C an 80 kR dose completely arrests induction and growth of the branchings. The growth reaction of fragments of *Drepanocladus aduncus* at 12°C after various radiation doses is illustrated by photographs 1a, b, c.

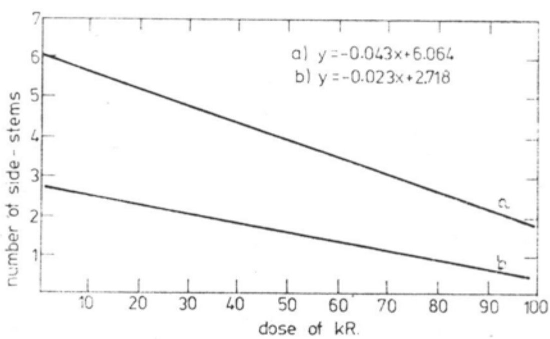

Fig. 9. Relation between number of side branchings of *Drepanocladus aduncus* (y) and radiation (x)
a — at 22°C, b — at 12°C

At 22°C growth ceased after a 160 kR dose (max.), whereas at 12°C growth no more occurred after 120 kR. A simple negative correlation was found between the growth of the main shoots of *Drepanocladus aduncus* and radiation; with increasing doses the elongation growth diminshes (Fig. 8). There also is a simple negative correlation between lateral branching induction and radiation; with increasing doses induction decreases (Fig. 9).

At 22°C and 12°C the lethal dose — LD 50 per cent for *D. aduncus* is 110 kR, but LD 80 and 100 per cent doses are lower at 12°C than at 22°C (Table 2). Dying of shoot fragments was observed as early as the second date in both experiments. Death occurred gradually. The first symptom was yellowing of the leaves, then of the branches, shedding of the growth apexes and finally complete fragmentation of the plants.

Table 2

Mortality of the moss *Drepanocladus aduncus*

Temperature	Lethal doses (LD) kR		
	50%	80%	100%
22°C	110	150	160
12°C	110	115	120

POSTIRRADIATION DEVELOPMENTAL ANOMALIES

In all experiments a weaker constitution of irradiated plants was noted. The plants are frail as compared with the control ones in experiments 2, 3 and 4. Transfer of the plants to culture does not affect their constitution. Irradiation caused diminution of the size of the leaves by as much as 2/3. Still greater diminution occurred at 22°C. Induction of side branchings caused by higher radiation doses causes further abnormal branching of the gametophyte mainly at the base of the growth apex (Photo 2a, b). Anomalous bushiness of the gametophyte is accompanied by a nonuniform development of side branchings of I order. In the natural *Drepanocladus aduncus* population from which the experimental plants were taken this type of branching was not observed; the side branchings of the gametophyte appear at regular distances and the corresponding ones show in general similar growth. In experiment 4 at 22°C with a dose of 130 kR complete chlorosis was observed in one of the side branchings. The remaining ones on this plant were normal.

RADIOACTIVITY OF PLANTS AND WATER

Alpha-, beta- and gamma-radioactivity of *Drepanocladus aduncus* plants from the natural population and the water from the microhabitat are shown in Table 3. The *D. aduncus* plants exhibit the highest alpha and the lowest beta activity. The alpha activity of the plants is as reported by Grodziński (1965), higher than the mean normal alpha activity of seed plants, it is, however lower than the mean normal alpha

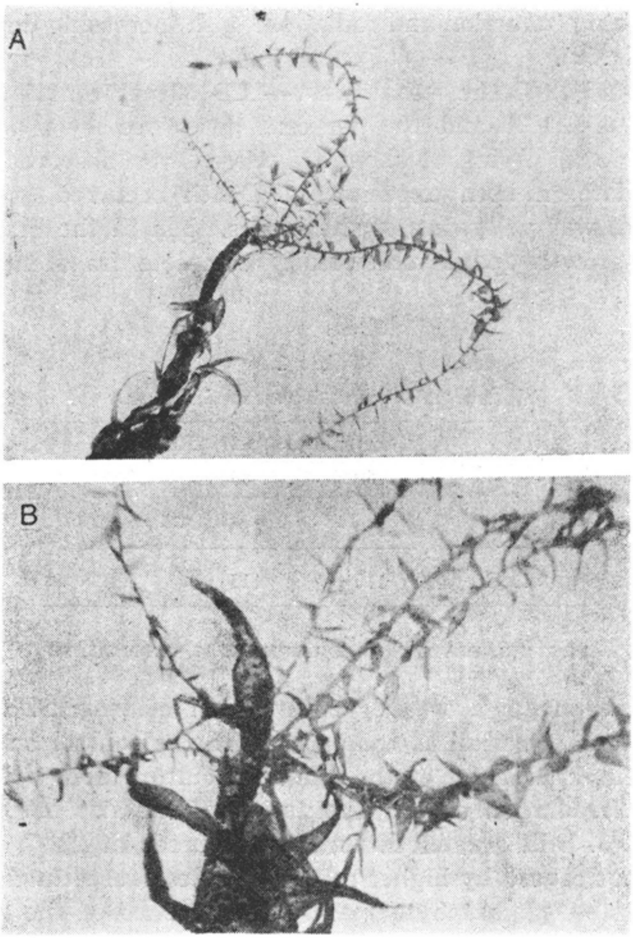

Photo. 2. Anomalous branching of gametophyte *Drepanocladus aduncus* a — owing to 125 kR dose at 22°C, b — to 155 kR dose at 22°C

Table 3

Radioactivity of the moss *Drepanocladus aduncus*

Sample		Radioactivity of ash (in cBq/g)				
		alfa	beta	gamma	Cs-137	Sr-90
Plants	1	830.65	7.51	280.83	5.18	2.96
	2	1038.59	21.20	301.92	10.73	4.44
	3	623.08	13.32	43.29	8.14	2.96
	$\bar{X}$	830.77	14.01	208.68	8.02	3.45
Water		—	7.51	51.43	2.96	1.85
Calibration constant=K		623.08	5.62	19.34	—	—

activity of land mosses. The plants of the examined population show a lower gamma activity than the mosses and liverworts growing on the areas of the Sudeten radiation anomalies (S a r o s i e k, 1972). Similarly, the natural beta activity of *D. aduncus* is about 10- to 100-fold lower that of land mosses (G r o d z i ń s k i, 1965; S a r o s i e k et al., 1973). The studied plants reveal the presence of radiostrontium and radiocesium, the amount of cesium being 2.5 times higher than that of strontium. Water from the microhabitat of the moss also contains these two isotopes. Water from this habitat is characterised by alpha activity within the limits of the background and an increased beta and gamma activity (Table 3). Radioactive water contamination of the locality Szczodre is low as compared with that of relatively uncontaminated waters of southern Poland (K w a p u l i ń s k i, 1975).

DISCUSSION

Similarly as in *Marchantia polymorpha* L. (S a r o s i e k, W o ż a k o w s k a - N a t k a n i e c, 1968) small doses of radiation stimulate in *Drepanocladus aduncus* elongation growth, whereas high doses inhibit it. This phenomenon may be explained according to S k o o g (1935) by the inactivation of auxin caused by radiation or else, after G o r d o n and W e b e r (1955), by inhibition of auxin synthesis the stronger the higher the radiation intensity. Growth stimulation of plants by low radiation doses was reported among others by M e r g e n and J o h a n s e n, 1964) and M c C o r m i c k and R u s h i n g (1964). The effect of growth stimulation in *Drepanocladus aduncus,* similarly as in *Marchantia polymorpha* occurs at relatively higher radiation doses (5 kR) than it does in higher plants. This gives sufficient grounds to consider that bryophytes are more tolerant to radiation. Inhibition of growth of the *D. aduncus* apical shoots due to higher radiation doses is associated with induction of lateral frequently irregular branchings. This branching may be considered as the typical postradiation reaction of bryophytes (S a r o s i e k et al., 1973). Lethal radiation doses for *Drepanocladus aduncus* (Table 2) indicate that this species is exceptionally resistant to acute gamma radiation, much more so than liverworts of the genus *Marchantia* (S a r o s i e k, 1972). If we know the chromosome number of *D. aduncus* (n=11 according to D a n i l k i v, 1976) and the volume of the cell nuclei (B r o d z k i, 1978) the radiosensitivity index (ICV) can be established according to the criteria of S p a r r o w (1962). For initial cells of the growth apex it is 9.00 μm^3, for cells of the segments close to the initial cell it is 2.54 μm^3, for cells of young leaf primordia it is 1.54 μm^3, for those in the growing internodes it is 1.69 μm^3 and for the cells of fully grown internodes 5.00 μm^3. The low values of this index correspond to the high resistivity of *Drepanocladus aduncus,* this being in agreement with the affirmation of Sparrow et al. (1968) that to higher index values corresponds a greater radiosensitivity of the plants.

Spectral analysis demonstrated that *Drepanocladus aduncus* plants from natural population have a relatively high calcium content in the ash and an increased beryllium content (0.001-0.01 per cent), lithium is also present. All these elements are in the opinion of Kalam (1970) important for the protection of plants against radiation. It may, therefore, be assumed that these nutrient components increase the resistivity of *D. aduncus* to radiation. Low temperature, on the contrary, in the present case 12°C, decreases the resistance *D. aduncus* to radiation (Fig. 1, 8). The value 12°C is beyond the ecological optimum.

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Promienioczułość mchu Drepanocladus aduncus (Hedw) Mnkm.

Streszczenie

Izolowane fragmenty gametofitu *Drepanocladus aduncus* w kulturach *in vitro* poddano ostremu promieniowaniu gamma z Co-60 o dawkach ekspozycji od 2 do 160 kR. Napromienione rośliny hodowano w temperaturze 12 i 22°C. Rośliny doświadczalne, pochodzące z naturalnej populacji ze stawu w Szczodrym k/Wrocławia, wykazywały podwyższoną radioaktywność promieniowania alfa, beta i gamma (Tabela 3). Zwierały w swym popiele Sr-90, Cs-137, dużo wapnia, berylu i lit.

Stwierdzono prostą ujemną korelację między wzrostem łodyżek głównych *Drepanocladus aduncus* a promieniowaniem; ze wzrostem dawek promieniowania obniża się wzrost elongacyjny (Rys. 8). Również stwierdzono prostą korelację między liczbą łodyżek bocznych a promieniowaniem; ze wzrostem dawek promieniowania obniża się indukcja łodyżek bocznych (Rys. 9).

W niższej temperaturze (12°C) promieniowanie silniej ogranicza rozwój. W tej temperaturze niższe są dawki letalne DL 80 i DL 100% (Tabela 2). Całkowita śmiertelność *Drepanocladus aduncus* w temperaturze 22°C wystąpiła przy 160 kR, a w temperaturze 12°C przy 120 kR. Mech *Drepanocladus aduncus* jest zatem rośliną o dużej odporności na promieniowanie. Odpowiada temu niska wartość jądrowego indeksu promienioczułości obliczona dla tej rośliny — ICV od 1,54 do 9,00 μm^3.