ISSN 0018-8166

HYDROBIOLOGICAL JOURNAL

VOLUME 50 NUMBER 5 2014

begell

New York • Connecticut

Volume 50, Issue 5 2014

HYDROBIOLOGICAL JOURNAL

V. D. ROMANENKO
EDITOR-IN-CHIEF

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New York • Connecticut

AIMS AND SCOPE

This journal publishes material translated from the Russian-language bimonthly publication *Gidrobiologicheskiy Zhurnal* containing original articles from leading scientists, mainly from the Ukraine, Russia, Belarus, Moldova, Poland, and other countries in hydrobiology and hydroecology. *Hydrobiological Journal* deals with freshwater and marine hydrobiology, aquatic flora and fauna, fish-husbandry hydrobiology, and ichthyology, aquaculture, sanitary, engineering and technical hydrobiology, ecological physiology and biochemistry of aquatic plants and animals, aquatic microbiology, toxicology and radioecology, hydroparasitology, and ecological hydrology and hydrochemistry. *Hydrobiological Journal* is a valuable publication for freshwater and marine biologists and chemists, limnologists, environmental scientists, oceanographers, and toxicologists

Hydrobiological Journal (ISSN 0018-8166) is published bi-monthly by Begell House, Inc., 50 North Street, Danbury, Connecticut, 06810 Phone: 1-203-456-6161, Fax: 1-203-456-6167. USA subscription rate for 2014 is $2091.00. Add $10.00 per issue for foreign airmail shipping and handling fees for all orders shipped outside the United States or Canada. All subscriptions are payable in advance. Subscriptions are entered on an annual basis, i.e., January to December. For immediate service and charge card sales, call (203) 456-6161 Monday through Friday 9 AM-5 PM EST. Fax orders to (203) 456-6167. Send written orders to Subscriptions Department, Begell House, Inc., 50 North Street, Danbury, Connecticut, 06810. You can also visit our website at www.begellhouse.com or http://dl.begellhouse.com/

Printed November 5, 2014

HYDROBIOLOGICAL JOURNAL

Volume 50 **Number 5** **2014**

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Hydrobiological Journal, 2014, Vol. 50, No. 5

Effect of Rest Period on Germination of the Common Reed Seeds from the Water Bodies of the Chornobyl Exclusion Zone†

N. L. Shevtsova, A.A. Yavniuk & D.I. Gudkov
Institute of Hydrobiology
National Academy of Sciences of Ukraine
Kyiv, Ukraine

Effects of chronic irradiation by low doses of ionizing radiation on viability of the common reed *Phragmites australis* (Poacea) from the water bodies of the Chornobyl exclusion zone were studied. Indexes of germinating capacity, germinative energy, viability of seedlings and survival rate of seeds depending on radiation dose were determined. Effect of the "rest period" was revealed, that is increase on average by 20–25% of germinating capacity and viability of seedlings of the plants, growing under the chronic irradiation, after 6–7 months of rest under absence of irradiation. Reliable correlation was stated between physiological state of the reed seeds and absorbed dose rate of the parental plant.

KEYWORDS: *common reed, Phragmites australis, water bodies of the Chornobyl exclusion zone, viability of seeds, chronic ionizing radiation, low doses.*

Introduction

Wide use of nuclear technologies in energetic, medicine and armament means active forming of one more global environmental factor – anthropogenic radionuclide pollution. Major nuclear accidents, occurred over the last decades in the nuclear power plants, demonstrated reality of the wide-scale contamination of both aquatic and terrestrial ecosystems by the artificial radionuclides. So, scientists have to solve numerous problems, associated with safe coexistence of human and biota under radionuclide contamination and irradiation by doses, exceeding natural radiation background.

Biological effects of chronic irradiation by low doses are expressed as changes of important population parameters like productivity, development and mortality of the organisms in natural

† Originally published in *Gidrobiologicheskiy Zhurnal*, 2014, Vol. 50, No. 3, pp. 85–96.

ISSN 0018-8166

conditions; they are studied insufficiently, especially in the aquatic ecosystems. As studies of the terrestrial ecosystems showed, prolong impact of low ionizing radiation can cause restructuring and impoverishment of biocenoses, increase of frequency of teratologic changes in populations, elementary genetic modifications in a sequence of broods of the irradiated organisms, etc. [2, 16, 19, 20]. Taking into account lability, plasticity, tolerance and genetic heterogeneity of populations, it can be supposed that their prolong occurrence under low-rate radiation launches mechanisms of selection, which favor adaptation to the modified environmental conditions [2, 3, 15, 19].

Previous studies showed that in gradient of the radionuclide contamination of the water bodies of the Chornobyl exclusion zone (ChEZ), in the root cells of the common reed *Phragmites australis* (Cav.) Trin. ex. Steud. frequency of chromosomal aberrations increased and they were of multiple character [24], this conformed to high rate of genetic instability. It is logical to suppose that this can also affect other important population characteristics, particularly reproductive.

The aim of this work was to study viability of the seed of the common reed, which grows under elevated radiation load.

Material and Method

Studies were carried out over the years 2009–2012 in the water bodies of different rate of radioactive contamination. Seeds of the reed were taken in closed and low-flowing water bodies, subjected to the long-term radioecological monitoring [5], located in the left-bank flood land (lakes Glyboke and Daleke) and right-bank (lake Azbuchin, Yanovskiy side-arm and cooling pond of the Chornobyl NPP – CP ChNPP) flood land of the Prypiat' River within ChEZ (in follows – ChEZ water bodies). Obtained data were compared with analogous parameters of seed of the plants from the water bodies with background radiation rate (Lake Verbne and Kyiv reservoir) (in follows – background water bodies).

Practically all populations of the reed in the ChEZ water bodies were formed before the Chornobyl accident, they are regularly monitored since 1988 [4, 12], except populations of the Azbuchin Lake and Yanovskiy side-arm, where they were formed in 2002–2004 after construction of the right-bank flood protection sand dam [10].

Reed heads were collected in the middle September – late October from three sampling sites (0.5 m^2) in each water body. In laboratory seeds were husked from the heads, cleared from flakes, packed into paper packets and stored in cool place. Further, depending on the experiment aim, seeds were germinated or remained for storage.

For assessment of viability of the reed seeds two experimental series were carried out. In the first series seeds were used after short latent period and sown in 20–30 days after sampling (LPs), in the second series seeds were used after prolong latent period, equal to period of winter rest – 6–7 months (LPl). In the latter case seeds were sown in April – May, this term corresponded to beginning of vegetation season in the water bodies of the northern Polissia Zone. Seeds were germinated in the Petri dishes, placed in the shelves with illumination 5–10 klux, at the temperature 20–22 °C, meeting randomization conditions. Experiments were trice replicated.

Seeds' viability was assessed by germinating capacity, germinative energy, germinating period (time of appearance of the first and last seedlings), vital capacity of seedlings and their survival rate. Germinating capacity is an essential characteristic of the seed; it determines their ability to form sprouts. Germinative energy characterizes simultaneity of the seedlings' appearance and indicates physiological homogeneity of the seed. It is expressed as portion of seeds, germinated on the 6th day. Seedlings' viability was calculated with account of germinated seeds at the stage of the first true leaf. Achievement of this morphogenesis stage indicates further viability of a seedling and start of the apical and rood meristem functioning [25]. Seeds' survival was accepted as ratio of initial number of seeds in the experiment and number of seeds survived to the end of the experiment. Results were statistically processed using standard methods of variation statistics and correlation analysis [9, 13] and MS Excel.

Results and Discussion

For the reed from the considered water bodies total absorbed dose rate from radionuclides of the water thickness, bottom sediments, incorporated in the plant tissues and of background sources was calculated [24]. According to calculations, plants of the left-bank flood land of the Prypiat' River get maximal absorbed dose – 4.9–13.6 μGy/hour, and plants of the background water bodies get the least (30–300 nGy/hour).

Analysis of the viability parameters of the reed seeds showed notable difference along gradient of the absorbed dose rate. Seeds of plants from the ChEZ water bodies after LPs were characterized by significantly prolonged germinating period, which correlated with dose load on the parental plant (Table 1). The last seedlings of the seeds of plants, taken in the background water bodies, appeared on the 9th day after sowing, of the seeds, taken in the Yanovskiy side-arm and CP ChNPP – on the 19th day, and taken in the Glyboke, Daleke and Azbuchin lakes – on the 26th day.

Time of the first seedlings appearance was not affected by the irradiation dose of the parental plant. Though on the 2nd day of the experiment germinated 3–5% of seeds from the background water bodies and 32–38% seeds from the ChEZ water bodies, except seeds from the Glyboke Lake (8%), where parental plants get maximal dose.

Germinative energy of the reed seeds from the ChEZ water bodies was 1.5–2.0 times lower than of seeds from the background water bodies, and germinating capacity and seedlings viability was lower by 20–25% (see Table 1). Dynamics of the seeds germination also significantly differed (Fig. 1, *a*).

From 3rd to 5th maximal portion (on average 80%) germinated of seeds of the background water bodies. Curve of the seeds germinating was of standard *s*-shape, character for cultivated and wild cereals [1, 11]. Different pattern was registered in plants of the water bodies with elevated radiation background – curves of germination were described by lineal function and were straight lines with tangent of incidence to the X-axis in diapason 0.8–2.2. This indicated durability of the seeds germination and, consequently, physiological heterogeneity of the seeds. Tangent of the straight lines incidence correlated with dose rate ($r = 0.89$), so physiological state of the reed seeds after the short latent period depended on the dose rate.

Table 1

Viability parameters of seeds and seedlings of the common reed after short latent period ($M \pm m$)

Water bodies	Average absorbed dose rate µGy/hour	Time of seedlings appearance, day		Germinating capacity, %	Germinative energy (5th day), %	Seedlings' viability, %
		the first	the last			
			ChEZ			
Glyboke Lake	13.64 ± 0.61	2	26	60.00 ± 1.41	14.57 ± 2.12	63.33 ± 1.14
Daleke Lake	5.99 ± 0.45	2	26	59.33 ± 0.62	44.00 ± 0.92	59.55 ± 0.68
Azbuchin Lake	5.02 ± 0.24	2	26	66.67 ± 1.84	45.33 ± 0.96	63.00 ± 1.18
Yanovskiy side-arm	4.17 ± 0.28	2	19	55.33 ± 0.93	42.00 ± 1.01	66.27 ± 1.21
CP ChNPP	2.53 ± 0.11	2	19	72.67 ± 1.15	56.67 ± 0.78	76.15 ± 1.31
			Background			
Kyiv reservoir	0.30 ± 0.01	2	9	83.33 ± 7.31	77.33 ± 0.57	92.00 ± 4.37
Verbne Lake	0.03 ± 0.05	2	9	92.67 ± 1.63	82.00 ± 0.34	95.68 ± 1.82

According to the literature data [8, 11, 17] seeds of the cultivated cereals mainly are not able to germinate immediately after harvesting. After-harvest ripening is a biochemical process, which leads to physiological maturity of the seeds, that is to their ability to produce normal seedlings. Its duration depends on species and climatic conditions of its habitat [1, 11]. Till the end of the ripening seeds have low germinating capacity or do not germinate at all. Minimal rest period, sufficient for seeds of the wild cereals to achieve physiological maturity, amounts to 3–14 days [1, 11, 14]. There is information [6, 23] that the reed seeds do not need ripening at all, and the main factor to limit autumn germination is unfavorable soil temperature [11]. Obtained results enable to suppose (see Table 1), that one month rest period is sufficient for the reed seeds to achieve physiological maturity.

In the temperate zone dissemination of the reed starts in September [14]. Seeds are scattered by wind and fall on soil, water, quagmires, etc. They start to germinate in spring at the soil temperature 16–18°C [6]. Vegetation period of the reed in the temperate zone starts not earlier than late April [14]. So, in the Polissia zone natural rest period of its seeds amounts to 6–7 moths. Their germinating capacity in spring is quite low – 35–55% [6, 23]. In nature and in artificial water bodies the reed mostly propagates asexually by underground stems [7, 8, 14].

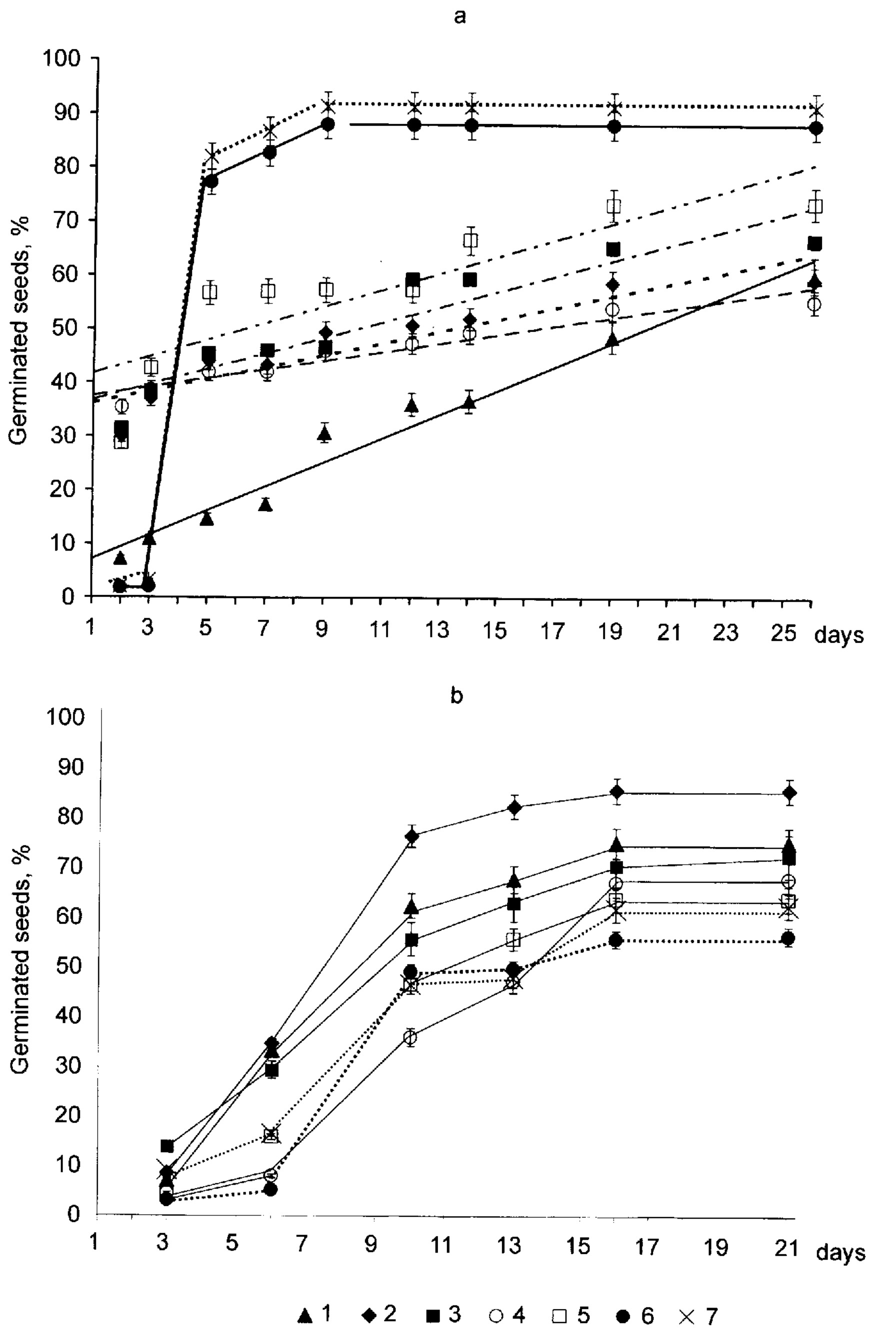

Fig. 1. Dynamics of germination of the common reed seeds, taken in the water bodies with different dose rate on the parental plant: *a* – short latent period; *b* – long latent period; *1* – Glyboke Lake; *2* – Daleke Lake; *3* – Azbuchin lake; *4* – Yanovskiy side-arm; *5* – CP ChNPP; *6* – Kyiv reservoir; *7* – Verbne lake.

Viability p

Water bodies
Glyboke Lake
Daleke Lake
Azbuchin Lake
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Table 2

Viability parameters of seeds and seedlings of the common reed after long latent period ($M \pm m$)

Water bodies	Average absorbed dose rate μGy/hour	Time of seedlings appearance, day		Germinating capacity, %	Germinative energy (5th day), %	Seedlings' viability, %
		the first	the last			
			ChEZ			
Glyboke Lake	13.64 ± 0.61	3	21	75.33 ± 2.35	33.33 ± 1.66	89.38 ± 3.38
Daleke Lake	5.99 ± 0.45	3	21	86.00 ± 3.27	34.67 ± 1.89	87.59 ± 3.22
Azbuchin Lake	5.02 ± 0.24	3	21	72.67 ± 2.28	29.33 ± 1.18	83.96 ± 3.30
Yanovskiy side-arm	4.17 ± 0.28	3	21	68.00 ± 1.15	8.00 ± 1.02	92.16 ± 3.95
CP ChNPP	2.53 ± 0.11	3	16	64.00 ± 1.43	16.00 ± 1.66	80.21 ± 2.94
			Background			
Kyiv reservoir	0.30 ± 0.01	3	21	56.67 ± 1.07	5.33 ± 1.16	89.41 ± 2.15
Verbne Lake	0.03 ± 0.005	3	21	62.67 ± 1.93	16.67 ± 1.33	92.55 ± 3.12

So, the series of experiments were carried out, where seeds were sown after latent period 6–7 months long (LPl), which corresponds to natural rest period. After long rest period differences of viability parameters between the reed seeds from the ChEZ water bodies and background water bodies decreased (Table 2). Germinating capacity of seeds from the ChEZ water bodies increased by 10% and on average amounted to 73%, seedlings' viability increased by 20% – on average to 87%, and germinative energy decreased by 16%.

Different pattern was observed in the reed seeds from the background water bodies: after long rest period their germinating capacity decreased by 238%, and germinative energy decreased almost seven times and was equal on average to 11%. However, viability of the appeared seedlings practically did not change and was equal on average to 91% (compare: 94% at LPs).

So, for the first time we revealed the effect of growth of the germinating capacity and viability of the seedlings of the plant of the fam. Poacea (cereals), vegetating under long-term chronic irradiation, after prolong rest period under background irradiation.

Table 3

Survival rate of the reed seeds depending on duration of the latent period

Water bodies	Average absorbed dose rate μGy/hour	Survival rate, %	
		LPs	LPl
ChEZ			
Glyboke Lake	13.64 ± 0.61	38.00 ± 1.14	67.33 ± 0.95
Daleke Lake	5.99 ± 0.45	35.33±0.68	75.33±0.66
Azbuchin Lake	5.02 ± 0.24	42.00 ± 1.18	61.01 ± 1.34
Yanovskiy side-arm	4.17 ± 0.28	36.67 ± 1.21	62.67 ± 0.98
CP ChNPP	2.53 ± 0.11	55.33 ± 1.31	51.33 ± 1.01
Background			
Kyiv reservoir	0.30 ± 0.061	76.67 ± 1.42	50.66 ± 1.12
Verbne Lake	0.03 ± 0.005	88. 67 ± 2.37	58.98 ± 0.93

It is worth to note that information on ability of the reed seeds to preserve germinating capacity in the course of time is quite contradictory. Some specialists indicate that they preserve germinating capacity for four and more years [6], others consider them to lost ability to germinate already in one year [8, 23]. However it is known [6, 7, 8, 14], that in nature the reed usually propagates itself asexually, and propagation by seeds needs coincidence of many favorable factors and occurs quite rarely [8, 14, 23]. It can be supposed, that considerable decrease of viability and especially germinative energy of the reed seeds from the background water bodies after long (winter) rest period, the most probably indicated natural aging of the seeds [11, 22].

At LPl dynamics of the seeds germination in all variants conformed to the linear-quadratic function and was described by *s*-shaped curve independently on the dose load on the parental plant (see Fig. 1, *b*). The first seedlings appeared on the third day, and period of germination amounted to 21 days (except seeds from the CP ChNPP) and did not depend on the origin of the parental plant. So, prolong stay in the rest state under absence of chronic irradiation led to equalization of physiological state of the reed seeds from the water bodies with different rate of radionuclide contamination, this was also confirmed by the literature data [1, 17, 18, 22]. At this viability of the seeds from the background water bodies decreased on average by 25%, and in seeds from the ChEZ water bodies it increased by 20% (Table 3).

It is worth noting that survival rate of seeds of plants from the ChEZ water bodies after long latent period, and from the background water bodies after short latent period, was quite close (see Table 3). So, value of 60–80% can be considered as conditional norm for these water bodies.

Decrease of survival rate of the reed seeds from the background water bodies after LPl on average by 25% the most probably was connected with loss of ability to germinate because of simple ag-

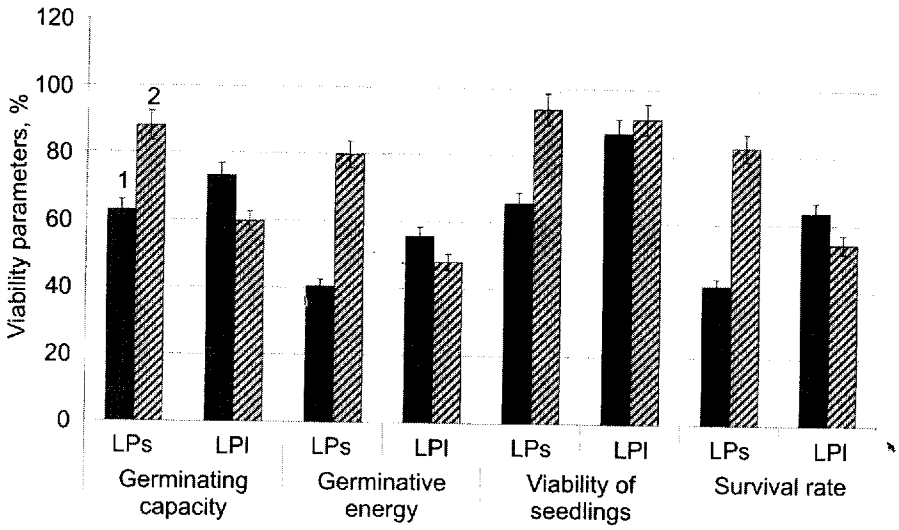

Fig. 2. Parameters of viability of the seeds of the common reed from the ChEZ water bodies (*1*) and background water bodies (*2*): *LPs* – short latent period: *LPl* – long latent period.

ing [1, 17, 23]. This assumption was confirmed by stably high viability of the seedlings – on average 90% as compared with 94% at LPs (Fig. 2). At the same time growth of the survival rate of the seeds from the ChEZ water bodies after LPl was supported by increase of all viability characteristics (see Fig. 2), and this probably confirms to functioning of the mechanisms of stress-protection and reparation systems [3, 17, 22].

It can be supposed that main part of biochemical, genetic and physiological disturbances, responsible for viability of the reed seeds from the ChEZ water bodies, was caused by cumulative impact of doses of chronic radiation. Over the short latent period after stop of the dose load regulatory systems of a germ do not have time to compensate all accumulated disturbances. This is realized in significantly decreased viability parameters of the seeds from the ChEZ water bodies as compared with seeds from the background water bodies. Over the induced rest period genome of the meristem cells of the germ is in partially depressed state, which is particularly controlled by the stress-proteins [22]. Prolong rest period, which is characterized by decelerated, but not stopped metabolic processes [1, 22, 25], under absence of irradiation creates preconditions for elimination of some disturbances, accumulated over life period of the parental plant under elevated dose loads. Increase (on average by 20%) of the germinating capacity of seeds after LPl enables to suppose that the main part in this process is assigned to reparation of genes', controlling synthesis of phytohormones, responsible for the seeds' germination. It is also supposed that intensification of reparation of the genome disturbances in the germs of seeds of the plants from ChEZ water bodies also leads to elimination of disturbances, responsible for aging. Besides, increase of the seedlings' viability on average by 25% can be connected with normalization of reparation of serious DNA damages – two-thread breaks. So, revealed effect of the "rest period" is expressed as increase of the survival rate of the seeds from the ChEZ water bodies due to complicated not-specific mechanisms of the ontogenetic adaptation to the prolong impact of the radiation factor.

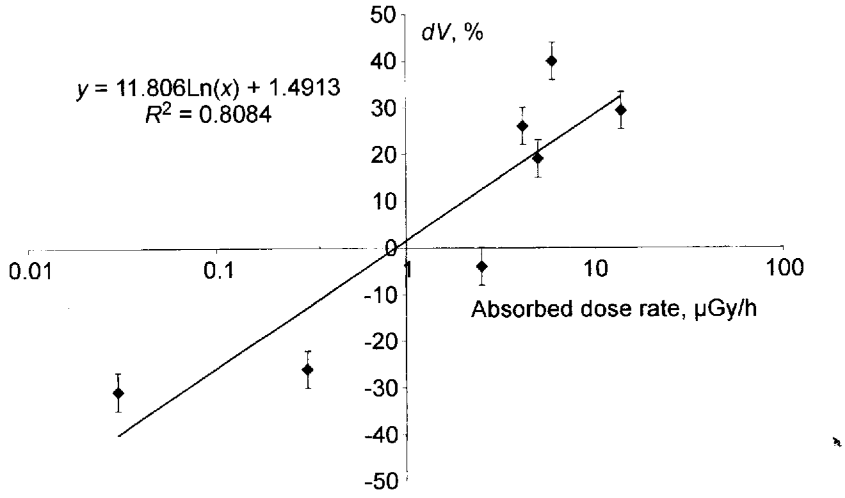

Fig. 3. Dose dependence of the "rest period" effect of the seeds of the common reed: *dV* – difference between survival of the seeds after long and short latent period.

In spite of the fact, that no direct correlation between survival rate of the reed seed and radiation absorbed dose of the parental plant was observed, the indirect dose dependence was revealed. High correlation score was noted between the dose load on the parental plant and difference of the seeds' survival rate after short and long latent period (correlation coefficient $r = 0.81$) (Fig. 3).

Conclusion

Studies revealed differences in viability of the seeds of the common reed from the water bodies of the Chornobyl exclusion zone with different rate of radionuclide contamination and background water bodies.

The month period after harvesting of the seeds in October is sufficient for physiological ripening of the common reed seeds.

Germinating capacity of the physiologically-mature seeds of the reed from the background water bodies is equal on average to 80%, and viability of seedlings – to 90–95%. Prolong period of induced rest negatively affects germinating capacity of these seeds – it decreases from 83–93% to 57–63%, however viability of seedlings practically does not change. Survival of the seeds decreased from 75–90% to 50–60% after prolong (6–7 months) rest period under background radiation level.

Seeds of the common reed from the water bodies of the Chornobyl exclusion zone with elevated dose loads on the parental plant were characterized by low viability parameters. Germinating capacity varied within the limits 55–73%, and seeds viability – 60–76%. Survival rate of the physiologically mature seeds amounted to 35–55%.

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For the first time the "rest period" effect was revealed, that is increase of the germinating capacity (on average by 20–25%) and viability of the seedlings of the plant of the fam. Poacea (cereals), vegetating under long-term chronic irradiation, after 6–7 month of rest under background irradiation. Rate of germinating capacity and viability of the seeds from the water bodies of the Chornobyl exclusion zone after long rest period was shown to be close to those of the plants from the water bodies with background radionuclide contamination after short rest period.

Reliable correlation was found between physiological state of the common reed seeds and absorbed dose rate of the parental plant.

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