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Effects of X-rays on seed germination and growth of Scots pine (*Pinus silvestris* L.) seedlings of different provenience

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Abstract:

The effect of ionizing radiation on germination of seed and growth of seedlings of Scots pine was investigated. Higher radioresistance of seed originating from the highland region was established. No difference was found in the investigated seed samples in the content of cysteine and content and composition of the chloroform-soluble fraction. Differences in compounds contained in the chloroform-soluble fraction and in the rate of phosphorus uptake were found in young seedlings of different provenience. They possibly are related to the difference in the ability of seedlings to regenerate the postirradiation damage.

INTRODUCTION

Adaptation of living organisms to a higher level of biosphere radiation is a result of evolutionary selection of form with increased resistance to irradiation (Dubinin, 1961). It has been found, that in many cases higher resistance to irradiation is connected with better tolerance of ultraviolet radiation (Grodziński, 1965). The above mentioned ecological factors change greatly with altitude and latitude (Piercow, 1964). Therefore it can be expected that plants belonging to the same species growing in different ecological conditions will show phylogenetically established adaptation to different levels of irradiation (Mc Cormick, 1964).

The main purpose of the here described experiments was to compare the radiosensitivity of Scots pine seeds of different provenience. The content of some compounds which could influence their radiosensitivity was also determined.

MATERIAL AND METHODS

Pine seeds (*Pinus silvestris* L.) from three different seed sources (highland: Nowy Targ and lowland: Spała and Dłużek) were irradiated with increasing doses of X-rays administered by a Picher Ferranti machine. The conditions of irradiation were as follows: 80 kV and 12 mA, intensivity of 1 Kr/min, temperature 18—20°C.

This research was carried out with the financial assistance of the U.S. Department of Agriculture.

Seeds after various periods of storage were used for investigation: (i) seeds from 1964/65 harvest — five years old, (ii) seeds from 1968/69 harvest — six months old.

The five years old seeds contained 5,1 % of moisture, and those six months old — 5,6 % of moisture. The number of seed was 80—120 in each experimental variant. The experiments were replicated three times at least. In the case of investigation on the influence of moisture content in seeds on radiosensitivity, the seed moisture was increased by storage in an atmosphere with high moisture content, or decreased by drying them in a vacuum 10^{-1}T.

The seeds after irradiation were sown on thick layer of wet cellulose paper and placed in a thermostat with constant temperature of 24°C and illumination of 1000 lux. The number of germinated seeds was determined after 8 and 21 days. After 21 days the seedlings were sampled and the length of the seedling, number of needles, fresh and dry weight of needles and stems were determined.

Young seedlings in the stage of cotyledons development were transferred to Ingestad's medium (Ingestad, 1962/63) recommended for water culture of pine; the plants were grown in greenhouse in daylight extended to 16 hrs by fluorescent lamps providing 2500 lux illumination. After six weeks of growth the dry weight of needles, roots and stems was determined. Besides, an additional experiment was carried out: nine plants taken from the control set, 1 Kr and 2 Kr (three plants in three replications) were placed with their root systems in ten times diluted medium containing $NaH_2{}^{32}PO_4$. After one hour of exposure, the plants were sampled and dry weight of their roots, stems and needles was determined. Each part was homogenized and radioactivity was determined with a GM tube having a mica window 1 mg/cm^2.

Free aminoacids were extracted with 80 % ethanol and separated by thin-layer chromatography (MN Cellulosepulver 300) with buthanol:acetic acid: water (4:1:1 v/v) as solvents.

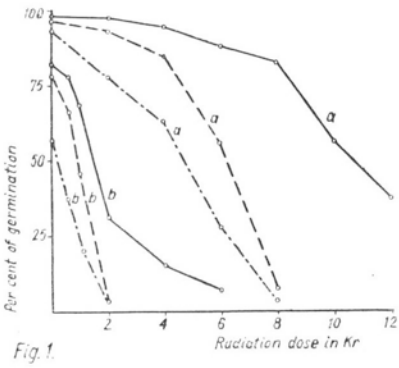
For spectral infrared analysis 1 g of lyophilized seeds and needles of 21-day-old seedlings were homogenized and extracted completely with chloroform; the solvent was later evaporated in vacuum at 5°—8°C. The residues were dissolved in 1 ml of chloroform, and 0.1 ml of solution was placed in the NaCl cell of a UR-10 (Zeiss Jena) spectrophotometer. Absorption was determined between 800—2100 cm^{-1} with the use of a NaCl prism and between 2100—3400 cm^{-1} with LiF prism.

RESULTS

Five-year-old and six-month-old pine seeds of each investigated seed sources exhibited a decreasing ability of germination with the increasing dose of X-radiation.

However, whereas a dose of 8 Kr almost completely stopped germination of a six-month-old seeds of provenience from Spała and Dłużek, seeds from Nowy Targ still germinated in about 30 % after a dose of 12 Kr (fig. 1a). LD_{50} of the

germination of the seeds of different provenience were respectively: Dłużek — 5 Kr, Spała — 6 Kr and Nowy Targ — 10 Kr. The results obtained for germination of five-year-old seeds showed also that seeds from Nowy Targ were almost twice more



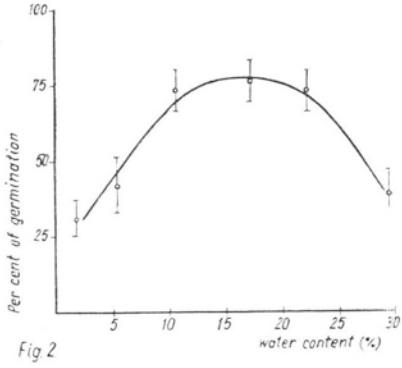


Fig. 1. Germination of seed in plants of different provenience: Dłużek —·—·—, Spała — — — and Nowy Targ ———
a) half year old seed, b) five years old seed

Fig. 2. Germination of seed of different water content irradiated with 4 Kr dose.

resistant to irradiation than those of other proveniences (Fg. 1b). Nevertheless five-year-old seeds of each provenience were about five times less resistant to irradiation than the six-month-old seeds.

The decrease in resistance to irradiation was not clearly correlated with the decrease of ability of germination typical for ageing of seeds. Also a slight difference in moisture content between both samples of seeds could not be responsible for the noted increase in sensitivity to irradiation of the five-year-old seeds. Proof of that may be found in the results presented in fig. 2 obtained from the experiment with six-month-old seeds from Spała. A decrease in seed moisture below 10% and an increase above 20% cause a rapid decrease in resistance to irradiation. The differences in moisture contents of 5.1% and 5.6% (that is the moisture content of the five-year-old and six-months-old seeds) are several times lower than the differences in moisture content of seeds in the experiment described above.

The growth of three-week-old seedlings obtained from seeds of different provenience, after irradiation with 0—4 Kr doses did not differ much (the differences were not proved statistically), either as regards fresh or dry weight of stems and cotyledons. The most marked differences were observed in the growth of juvenile needles, but these, in this stage of growth, constituted only about 20% of the whole plant. Wider differences were obserwed, however, in six-week-old seedlings grown from irradiated seeds in water culture conditions. All seedlings obtained from the five-year-old seeds from Nowy Targ exhibited better growth after the applied doses

of irradiation. The other two sets of seedlings from Dłużek and Spała reacted however to the same doses of irradiation by a decrease of growth by about 20% (Fig. 3).

Seedlings from Dłużek in this experiment died after treatment with 2 Kr. In the case of seedlings grown from six-month-old seeds, of each provenience they showed enchanced growth up to 1.5 Kr of irradiation (Fig. 4).

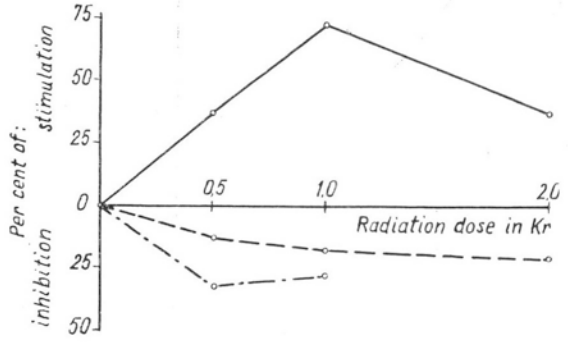


Fig. 3

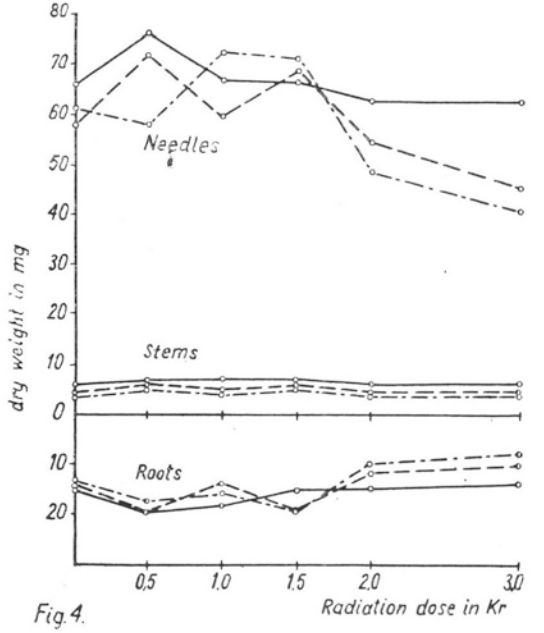


Fig. 4.

Fig. 3. Growth of seedlings in water culture conditions after irradiation of five years old seed c alculated in percent of control plants. Provenience: Dłużek —·—·—, Spała— — —, Nowy Targ———

Fig. 4. Dry weight of various organs of seedlings grown in water culture conditions after irra-. diation of half year old seed; provenience: Dłużek —·—·—, Spała — — —, Nowy Targ ———

When a dose of 3 Kr was applied, only seedlings from Nowy Targ did not react by less intensive growth, and the seedlings from Spała and Dłużek reduced growth by abouth 30%. The same parts of seedlings of different provenience reacted in a different manner to the same dose of irradiation. This caused, beside others effects, a change in the ratio of shoots to roots. This ratio was lowest for the plants of lowland provenience at 0.5 Kr, and for the highland plants at the dose of 1.0 Kr. The differences in dry weight noted in this experiment were caused almost exclusively by the differences in growth of juvenile needles and roots, because the reaction of cotyledons to the applied doses of irradiation was very slight.

Table 1

Dry weight of plants and accumulation of radioactive phosphorus in needles of 6 weeks old seedlings

Provenience	Dose	Dry weight (mg)			Activity in needles	
		root	stem	needles	total 10^3 cpm/plant	specific 10^3 cpm/g.dr.wt.
Nowy Targ	0	16,0	6,0	66.0	7.4	112
	1	15.7	6.0	66.3	8.4	127
	2	13.3	5.0	59.3	6.6	111
Spała	0	14.4	4.4	58.1	7.0	120
	1	16.9	4,4	59.3	6.4	108
	2	12.0	4.1	52.7	4.3	82
Dłużek	0	14.4	4.4	60.8	6.4	105
	1	16.9	4.3	73.0	6.8	93
	2	11.8	4.1	46.0	3.5	76

In an additional experiment some differences in the ability of uptake and accumulation of radioactive phosphorus in the needles of the investigated plants were noted. Such differences occured both between plants of different provenience and between samples of seedlings of the same provenience treated by various doses of X-rays (Tab. 1). The highest radioactivity of needles was noted in seedlings from Nowy Targ, what might be explained by their abundant roots and needles in this stage of growth. In plants originating from Spała and Dłużek differences in radioactivity were noted in spite of similar weights of their roots and needles. After a dose of 1 Kr an enchanced accumulation of phosphorus in the needles of plants from Nowy Targ and a decrease in phosphorus accumulation by about 10% (demonstrated by decrease of specific activity) in those of other provenience was observed. When the dose of 2 Kr was applied, in the seedlings of lowland provenience the accumulation of phosphorus decreased by about 30%, but in those from the highland provenience the accumulation was the same as in the control plants.

By thin-layer chromatography differences in the content and proportions of free amino acids between plants of different provenience in control as well as in irradiated seedlings were not noted. With increase of irradiation doses only a rapid

increase of free proline was observed. After a dose of 6 Kr, free proline content rose tenfold as compared with that in the control plants.

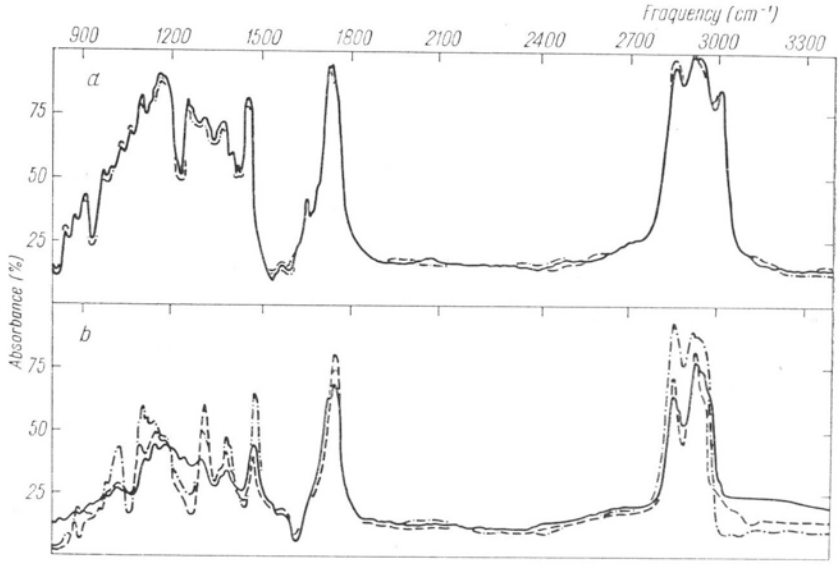


Fig. 5. Infrared absorption spectrum of chlorophorm fraction from seeds (upper part) and needles (lower part); provenience: Dłużek —·—·—, Spała — — —, Nowy Targ ———.

No differences in the infrared spectrum of the chloroform extracted fraction from all seeds were observed as regard the provenience comparison (Fig. 5a). On the other hand, clear differences in this respect were noted in two-week-old seedlings (Fig. 5b). The differences were marked by a decrease of maximum absorption at 920, 1170, 1740 and 3015 cm^{-1}. Particular emphasis should be put on the very high absorption at 1720 cm^{-1} in the case of seedlings extracts from plant originating from Nowy Targ. In the lowland plants this maximum, very intensive in extracts from seeds, and the same for the extracts of different provenience, is much less pronounced in extracts from seedlings. Besides, the maxima of absorption at 2860 and 2940 cm^{-1} in extracts from seedlings originating from Spała and Nowy Targ are lower than in those from Dłużek. Similar differences between the plants of different provenience were observed also in the case of extracts from needles of the four-year plants.

DISCUSSION

One of the causes, noted by many authors, of differences in growth of Scots pine (*Pinus silvestris* L.) of different provenience, could be their unequal sensitivity and reaction to the same external factors (Bourdeau, 1963; Białobok, 1967; Żelawski, 1968; Żelawski et al. 1968) as generally confirmed by the results of these investigations. The unequal sensitivity of seeds of different provenience to the

influence of the same dose of *X*-rays was manifest not only in seed germination, but also in the different rate of seedlings growth. The higher radioresistance of the seeds from the highland plants as compared with the lowland ones confirms similar results obtained in former investigation by other authors (Ohba and Simak, 1961). It is worth noting that the differences described are also observed in the case of five-year-old seeds, much more sensitive to X-rays than the six-month-old ones. The higher sensitivity of five-year-old seeds could be connected with the process of ageing (Nalborczyk, 1970), or may result from the influence of the different climatic and edaphic conditions during the formation and maturation of the seeds (Januszkiewicz, 1963).

The relationship between the moisture content and the sensitivity to irradiation of the pine seeds exhibits a similar pattern as is in the case of seeds of other plant species (Myttenaere et al., 1965; Gustafsson and Simak, 1958). Within the natural range of moisture content in air-dry seeds (5—10%), significant differences in their radiosensitivity are observed. Therefore in all studies of pine seed radiosensitivity special care should be taken to precisely determine their moisture content. Determination of the radiosensitivity of air-dry seeds may lead to erroneous conclusions that they exhibit high sensitivity to irradiation. The same seeds, containing 10—20% of moisture can be quite resistant to irradiation. The same is true of seeds of another gymnosperm species (Rudolph, Miksche, 1970). Very symptomatic for the examined plants is also the lack of significant differences in the rate of growth of the three-week-old seedlings from seeds irradiated with low doses of X-rays (0.5–2.0 Kr). Slight inhibition of growth of these plants was observed after a dose of 3 Kr. This probably results from the fact that at this stage of growth the part of the organs formed in the embryo before irradiation was relatively high, while the applied doses of X-rays did not effect significantly elongation growth. This may partially explain the lack of clear effects of radiostimulation in some investigations of 2–3-week-old pine seedlings (Suszka et al., 1960). Our investigations indicate that distinct radiostimulation of Scots pine seedlings can be only observed in the stage of intensive growth of juvenile needles. In the described experiments radiostimulation occurred in six-week-old seedlings, where the juvenile needles amounted to more then 50% of the whole dry matter of the plant. In experiments with older seedlings still higher radiostimulation was observed (Żelawski and Nalborczyk, 1971). In the case of spruce seedlings, radiostimulation was also highest in the epicotyl (Thas, 1967).

Similarly, as in the case of seed germination, seedlings of the highland provenience exhibited the highest resistance of growth to irradiation in the range of 2–3 Kr. However, the different reaction of seedlings of each provenience to doses of 0.5–1.5 Kr should be emphasized. In the case of five-year-old seeds these doses resulted in growth stimulation only in seedlings of highland provenience. In the case of six-month-old seeds, stimulation occurred in all plants of different provenience. However, in each of the three seed sources a different pattern of radiostimulation was obserwed.

The results of the additional experiment on the uptake by roots and accumulation in needles of radioactive phosphorus also indicate a different course of this process in

seedlings of different provenience. Only in seedlings of the highlands provenience the decrease in roots and needles weight following a dose of 2 Kr did not cause a depression of specific radioactivity of phosphorus accumulated in the needles. This could be one of the possible explanations of the higher ability in the highland pines to regenerate after irradiation damage. In the case of seedlings of lowland provenience, this dose probably affected the decrease of phosphorus uptake by the root system, or the rate of its translocation to needles.

Attempts to find any difference in the content of a possible radioprotector or donor of sulphydryl groups in the form of cystein (Bacq and Alexander, 1961; Nalborczyk, 1967) gave negative results. On the other hand the increase of free proline during the germination of seed irradiated with high doses of X-rays was observed. A similar increase of free proline was also observed in case of gremination of irradiated seeds of lupine (Nalborczyk, 1967) and several other species of plants (Nalborczyk, unpublished data), which may indicate the wide occurence of this phenomenon in the plants.

Taking into account the high content of lipids in pine seeds, and among them particularly unsaturated fatty acids (Suszka, 1967) which can form during irradiation toxic peroxides and free radicals (Bowen and Thick, 1961; Kudraszow, 1966), differences in their content could be also expected in the seeds of different provenience. The results obtained with chloroform extracts of lipids from seeds did not confirm this supposition. Attention should also be called to the existence of significant differences in the composition of analogous fractions isolated from needles. Clear differences in absorption in the "fingerprint" region, and especially in the range 1720 cm^{-1} and 1740 cm^{-1} reveal differences between plants of different provenience in the composition of fatty substances occurring in needles (Rao, 1963). This could indicate a differentiation of the metabolic pathways of lipids during the growth of seedlings (James, 1969; Erdtman, 1969). The observed fact might have been, however, the result of different distribution of the investigated substances among the individual plant organs or the varying degree of their binding into lipoprotein complexes insoluble in chloroform. This changes in the chloroform-soluble fraction similarly as the differences in phosphorus uptake are possibly related to the difference in ability of seedlings to regenerate the postirradiation damage.

The explanation of the observed facts requires further studies.

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Wpływ promieni Roentgena na kiełkowanie nasion i wzrost siewek sosny zwyczajnej (Pinus silvestris L.) różnego pochodzenia

Streszczenie

Badano wpływ promieniowania jonizującego na kiełkowanie nasion i wzrost siewek pochodzenia górkiego (Nowy Targ) i niżowego (Dłużek i Spała) sosny zwyczajnej. We wszystkich przypadkach stwierdzono wyższą promienioodporność nasion pochodzenia górskiego. W badanych nasionach nie stwierdzono różnic w zawartości cysteiny i związków rozpuszczalnych w chloroformie, mogących wpływać na zmianę ich promienioczułości. Stwierdzono natomiast różnice zawartości związków rozpuszczalnych w chloroformie oraz inny przebieg pobierania fosforu u siewek różnego pochodzenia. Obserwowane różnice we wzroście siewek wyrosłych z napromieniowanych nasion sosny różnego pochodzenia mogą więc wynikać nie tylko z różnej wrażliwości nasion na napromienienie, lecz także z odmiennego przebiegu procesów metabolicznej regeneracji popromiennej.