Effect of X- and γ-rays on Conifer Seed

Röntgen- och gammastrålningens inverkan på barrträdsfrö

by

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Introduction

With respect to forest seed the depressive or stimulative effect of ionizing radiation on germinability and development of seedlings is little studied. Most experiments described pertain to agricultural material. Although there are certain similarities between the various kinds of seed, it is necessary that the results of experiments with agricultural material are reappraised when dealing with conifer seed. This paper has the character of such a complementary investigation.

The following aspects have been particularly studied:

1. X- and γ-radiation effects on pine seed of various degrees of ripeness.
2. Influence of X-rays on spruce seed of various moisture content.

The effect of various kinds of radiation has been carefully investigated for barley, wheat, etc. A review of the literature reveals that the ripeness of seed is of great importance.

In this experiment the X-radiation was applied by means of the laboratory facilities at the Genetics Department of the Forest Research Institute (Plate 2).

In γ-radiation a cobalt-60 source located at the experimental field of the Genetics Department (Röskär) was used (Plate 3).

Exposure of pine seed of various ripeness to X- and γ-radiation

In northern Sweden pine seed often ripens poorly due to severe climate. X-ray photographs of such seed frequently reveal incomplete development of embryo and endosperm and a high frequency of poly-embryonal seeds. The investigation has shown that poorly developed embryo and endosperm cause low germinability. In southern Sweden and on the European continent pine seed ripens well; the embryo and the endosperm grow to full size and the germinability is high.

SIMAK, MÜLLER-OLSEN and GUSTAFSSON have classified pine seed on the basis of embryo development into five embryo classes, 0—IV, a brief description of which is given.

Embryo class	0:	Neither embryo nor endosperm (= empty seed).
	I:	Endosperm but no embryo.
	II:	Endosperm and one or several embryos none of which longer than half of the embryo cavity.
	III:	Endosperm and one or more embryos, the longest of which measures between half and three quarters of the embryo cavity.
	IV:	Endosperm with one fully developed embryo, completely or almost completely filling the embryo cavity. Undersized embryos rarely occur.
Endosperm class	A:	The endosperm fills the seed almost completely and absorbs the X-radiation well.
	B:	The endosperm fills the seed incompletely and it is often shrunken or deformed. The X-radiation absorption is less than that of class A seed.

According to this classification, seed of embryo class I—III is common in northern Sweden and seed of embryo class III—IV in southern Sweden.

These differences in embryo development are conditioned mainly by climate (SIMAK and GUSTAFSSON 1954) — less by heredity. In other cases, however, poor embryo development of seed is definitely caused by hereditary factors (PLYM FORSHELL 1953, EHRENBERG and SIMAK 1957).

In reports by C. EHRENBERG *et al.* (1955), as well as GUSTAFSSON and SIMAK (1956) it has been shown that the embryo classes differ with regard to their physiological behaviour. This raises the question of how seed of different embryo classes react to X- and γ-radiation of various dosage levels. The problem is dealt with in this paper.

Material: Pine seed used in the experiment originated from the area of Arvidsjaur and Jokkmokk (province of Lapland), lat. 65°—66° N and 150—400 m elevation. Seed crop: 1955/56. Distribution of the seed by embryo classes was as follows:

Embryo class:	0	I	II	III	IV
per cent	20	3	12	18	47
Endosperm: A					

By means of X-ray photography (Plate 1) the seed was separated into embryo classes II, III and IV. The weight of 1 000 seeds was:

Embryo class II: 1.9270 g; Embryo class III: 2.1148 g; Embryo class IV: 2.8382 g.

Equilibration (cf. L. EHRENBERG 1955 a): The seed was equilibrated for

5 days at 40 per cent humidity. The importance of this measure is reviewed in a following section of this paper.

X-radiation: 150 kV, 10 mA, focus 50 cm. Filter 0.5 mm Cu + 1.0 mm Al. During exposure the seeds were kept between two one-millimetre-thick plexiglass plates. Dosages applied: 75 r, 150 r, 300 r, 600 r, 1 200 r, 2 400 r were controlled by automatic dosimeter. Exposure times: 3.75, 7.50, 15, 30, 60, 120 min., respectively. [At a recent calibration of the X-ray apparatus it appeared that the automatic dosimeter used in the experiment probably showed too low a value for the radiation dosage (approximately 15 per cent). This discrepancy is not of a magnitude important for the interpretation of the results.]

γ-radiation: Dosages and exposures as above. This was achieved at a distance of 28.1 cm from the cobalt-60 source.

Design of experiment: Two replications comprising 50 seeds per embryo class and dosage (except dosage 2 400 r to which embryo class IV only was exposed).

Conditions of sowing and germination: Immediately upon radiation each seed was sown separately in sand at exactly 0.5 cm depth. The germination bed was kept moist during the whole experiment by means of a water-absorbing layer of filter paper (Plate 4). The intensity of artificial light was approximately 1 000 Lux at the surface of the germination bed (12 hours/day). Room temperature: approximately 20° C. Every day the seed-beds were controlled and the germinated seedlings marked with a ring. The experiment was terminated after 50 days and for each embryo class and dosage the following data were computed:

1. Germinability after 50 days.
2. Rate of germination.

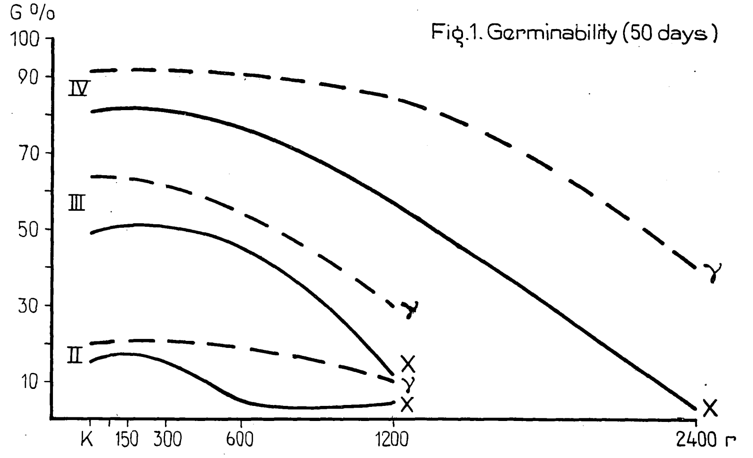


Fig.1. Germinability (50 days)

Results:

Germinability (Fig. 1).—The difference in germinability between the embryo classes was significant—embryo class II germinated poorly, embryo class IV excellently.

Germinability of the control series (no radiation) was as follows:

Embryo class	X-series	γ-series
II	15 per cent	22 per cent
III	51 »	66 »
IV	82 »	92 »

The difference between the X- and γ-series was due to the more favourable germination conditions for the γ-series (the two sets of series had separate germination beds). The slightly stimulative effect at low dosages of X- and γ-radiation indicated in Fig. 1 is insignificant statistically. At radiation intensities below 300 r the germinability of X-rayed seed may be considered unaffected in all the embryo classes. A depression is obvious after a radiation of 300 r or more. For γ-radiation the relationships are analogous although the depression starts later. The depression may be elucidated by means of DL_{50}, *i.e.* the dosage at which the germinability is reduced by 50 per cent in relation to that of control seed. The DL_{50} values summarized below are obtained by interpolation in Fig. 1:

Embryo class	X-radiation	γ-radiation
II	500 r	1 200 r
III	1 000 r	1 300 r
IV	1 500 r	2 300 r

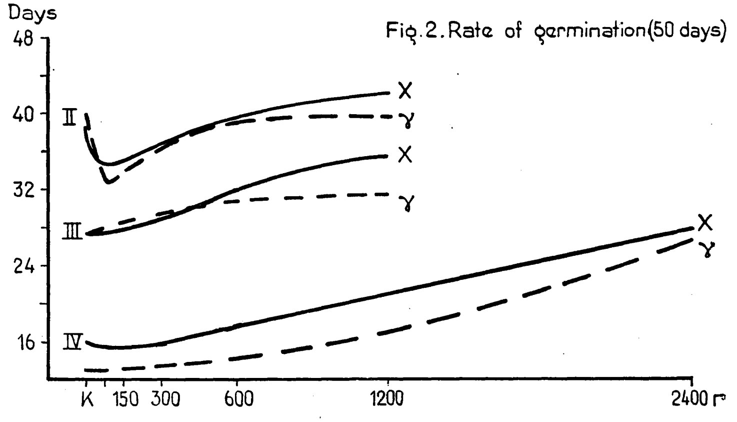
As will be seen the inhibitory effect of γ-radiation is generally less than that of X-rays, which also is obvious from the fact that seed of embryo class II is but twice as sensitive as that of embryo class IV. Corresponding relationship for X-radiation is three to one.

When comparing the biological effects of X-rays with those of γ-rays in this experiment the fact must be taken into consideration that the germination conditions of the irradiation series were different and the dosage values of X-radiation, given above, possibly somewhat too low.

Rate of germination[1] (Fig. 2).—The differences in the rate of germination between various embryo classes are significant for each of the control series:

Embryo class	X-series	γ-series
II	38 days	40 days
III	27 »	27 »
IV	16 »	13 »

[1] Rate of germination = weighed average number of days in germinator before germination occurs.



Any great differences in the rate of germination between the control series have not been noticeable. A stimulative effect of X-rays on the rate of germination is discernible only for seed of embryo class II, the germination of which is hastened by four days at a dosage of 75 r. For seed of the same embryo class this dosage of γ-radiation improves the rate of germination by seven days. The effect, however, is not very certain due to the low number of seedlings developed from seed of embryo class II.

A depression effect becomes discernible for seed exposed to X- and γ-rays at a dosage of 150—300 r. For seed exposed to γ-rays the depression is less than that for seed in the X-series (for seed of embryo class II no depression at all).

Reduction of the average rate of germination at 1 200 r dosage in relation to the control series:

Embryo class	X-radiation	γ-radiation
II	5 days	0 days
III	8 »	2 »
IV	5 »	4 »

As far as rate of germination is concerned, it appears that seeds of high embryo class are more sensitive than those of low embryo class. The most plausible reason for this phenomenon seems to be that a high dosage destroys poor seeds of the low embryo classes whereas seeds of high embryo classes are affected only with respect to vigour (compare germinability). Thus, the rate of germination of seed of low embryo classes is favoured by a selective effect of radiation.

Effect of X-radiation on spruce seed of various levels of moisture content

Moisture content of seed depends on the external conditions of seed storage *e.g.* air humidity, air temperature and the anatomic constitution of the seed (BARTELS 1956).

Moisture content of seed is of decisive importance for many physiological processes. The germination occurs only at a high moisture content of the seed, whereas a good storage, maintaining the seed vigour over a long period of time (GUSTAFSSON 1937 b, TOOLE 1953, HUSS 1954), requires a low moisture content. In addition, at low humidity of the air, seed tolerates much higher temperature than at high humidity (HERMELIN 1958). For pine seed from northern Sweden SIMAK and GUSTAFSSON (1957) showed in their investigations that underdeveloped embryos may be induced to grow at low temperature and 100 per cent air humidity. This treatment, called equilibration, is fundamentally a stratification process. It raises the germinability of seed considerably.

At the experiments with ionizing radiation, too, it has appeared that the moisture content of seed, particularly agricultural seed, is of great importance for the subsequent germination (L. EHRENBERG 1955 b).

The problem has been investigated for spruce seed and the results will be discussed here.

Material: Spruce seed used originated from the isle of Gotland, lat. 57°30′ N and 20 m elevation. Seed crop: 1956/57. The experiment comprised seed of embryo class IV A, which was separated by means of X-ray photography. The average weight of 1 000 seeds for the experimental series was 5.5408 g.

Equilibration: The seeds were exposed to radiation at three levels of moisture content corresponding to an air humidity of 0, 40 and 100 per cent. The air humidity desired was created in an equilibrator (L. EHRENBERG 1955 a). An air humidity of 0 per cent was achieved by forcing air through sulphuric acid and blue vitriol; 40 per cent humidity was achieved by forcing air through a potassium hydroxide solution of suitable concentration and 100 per cent air humidity was obtained by air passing through water. Air prepared this way was led through desiccators with seed contained in plastic tubes for seven days, until equilibrium between seed moisture content and air humidity was achieved. In a parallel control series the moisture content at various humidity levels was determined as weight in relation to dry weight (105° C):

Relative air humidity, per cent	Seed moisture content, per cent
0	2.82
40	5.94
100	25.40

X-radiation: 150 kV, 10 mA, focus 50 cm. Filter: 0.5 mm Cu + 1.0 mm Al. During radiation exposure the seed was stored in well closed plastic tubes used at the equilibration. Humidity in the plastic tubes did not change during radiation. The following levels of dosage were applied:

100 r, 200 r, 300 r, 600 r, 1 200 r and 2 400 r.

Design of experiment: 2 × 50 seeds per dosage and humidity level.

Conditions of sowing and germination: As in previous experiment. The effect of radiation was investigated with respect to:

1. Germinability.
2. Rate of germination.
3. Number of cotyledons developed.
4. Hypocotyl length.
5. Fresh weight of healthy seedlings.
6. Dry matter content of seedlings.
7. Mortality of seedlings.
8. Condition of ungerminated seeds.

Results:

Germinability (Fig. 3).—No stimulative effect would be detectable, since a germinative capacity of 98—99 per cent was recorded for the control material. Seed subjected to 0 per cent air humidity showed a germinability equalling that of the control seed at dosages below 300 r. Above this intensity of radiation a depression appeared and already at 1 200 r the germinability was 8 per cent after 50 days. After a radiation of 2 400 r no seeds germinated. Seed subjected to 40 or 100 per cent air humidity, however, germinated normally after radiation dosages below 1 200 r, *i.e.* germinability equalled that of the control seed. Above 1 200 r the depression started but slowly, and after 2 400 r the germinative capacity of the seed was still 80 per cent.

Rate of germination (Fig. 4).—No stimulative effect of radiation on the rate of germination was observed for any of the humidity levels applied. For seed subjected to 0 per cent air humidity a depression appeared after a radiation of 200 r and it continued quite rapidly at increasing dosage of radiation. For seed subjected to 40 or 100 per cent humidity the depression was very slight.

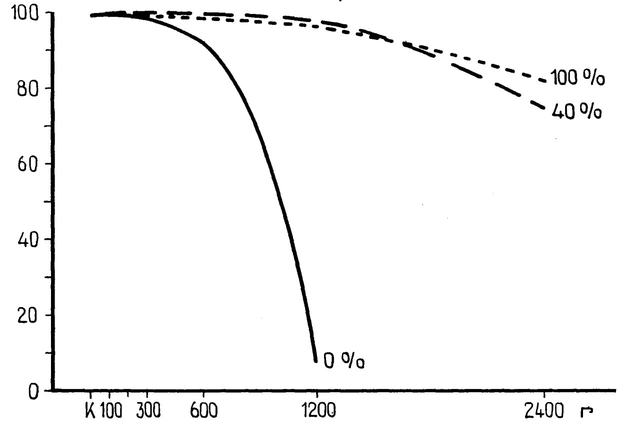


Fig. 3. Germinability (50 days)

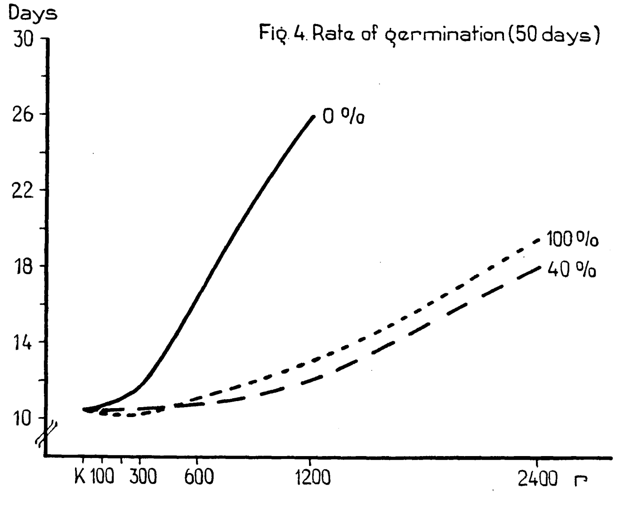


Fig. 4. Rate of germination (50 days)

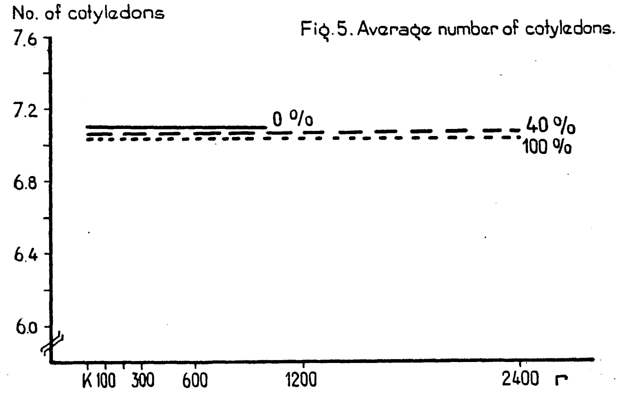


Fig. 5. Average number of cotyledons.

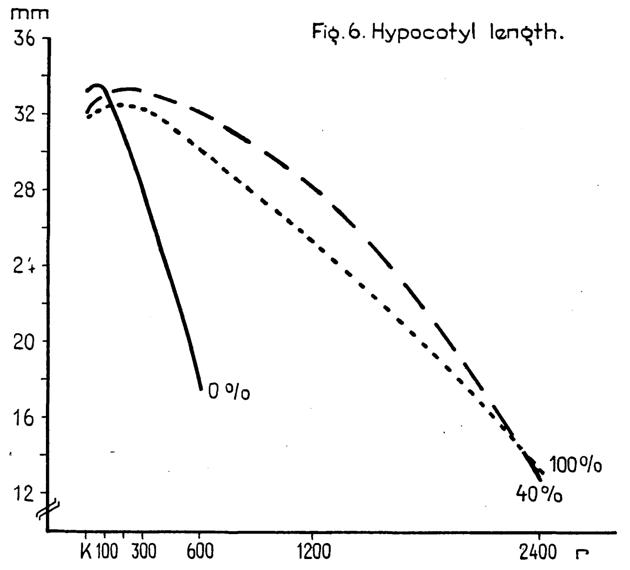


Fig. 6. Hypocotyl length.

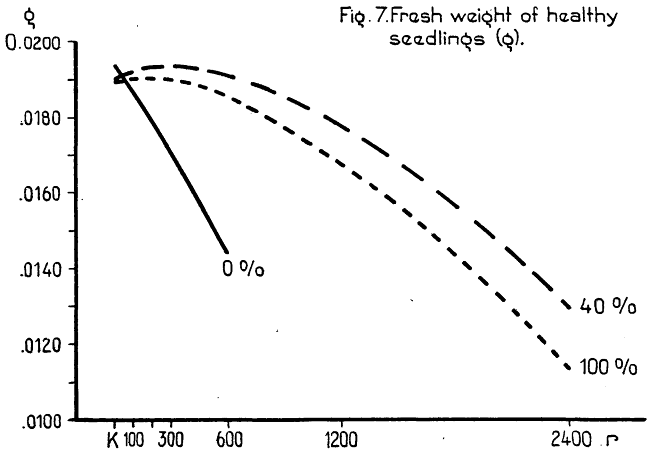


Fig. 7. Fresh weight of healthy seedlings (g).

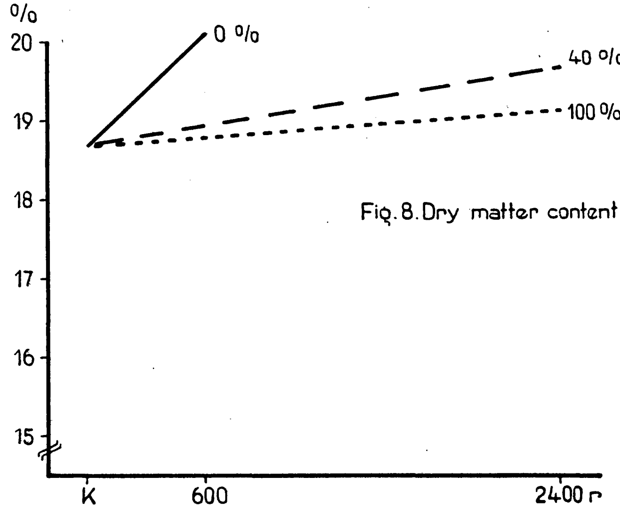


Fig. 8. Dry matter content (%)

Number of cotyledons developed (Fig. 5).—For each seedling the number of cotyledons was observed. The graphs show that the number of cotyledons remained the same all through various intensities of radiation and all levels of humidity (average circa 7).

Hypocotyl length (Fig. 6).—Thirty days after germination each seedling was cut and the hypocotyl was measured. By this individual cropping the differences in rate of germination at various intensities of radiation and humidity levels were eliminated. The slightly stimulative effect as to length of hypocotyl, indicated in Fig. 6, is not significant. Seed subjected to 0 per cent air humidity showed a depression already after 100—200 r. For seed subjected to 40 or 100 per cent air humidity the depression did not appear, until an intensity of radiation of 600 r and 300 r, respectively, was reached.

Values of 50 per cent decrease in hypocotyl length were obtained by interpolation of the dosages. (Average length of hypocotyl for the control series was approximately 33 mm.)

Air humidity	DL_{50}-value
0 per cent	Approximately 600 r
40 »	» 2 200 r
100 »	» 2 200 r

It should be pointed out that the seedlings developed from seed exposed to high radiation dosages are not only very stubby but also heavy and succulent. Frequently, deformities were observed on the whole of the seedling, *e.g.* short and irregularly developed cotyledons. In some cases the colour was dark green or the seedlings exhibited pale green spots.

Fresh weight of healthy seedlings (Fig. 7).—The seedlings cropped each day were weighed at an accuracy of 10^{-4} g. The average weight of seedlings within each series is shown graphically in Fig. 7. Radiation had no discernible stimulative effect on the weight of seedlings. For seed subjected to 0 per cent air humidity depression occurred already at the lowest dosage used, 100 r. For seed subjected to 40 or 100 per cent air humidity the depression appeared about 600 r. The reduction of seedling weight induced by 600 r for seed subjected to 0 per cent air humidity amounted to 25 per cent. Corresponding treatments of 40 and 100 per cent air humidity did not cause any significant reduction of the seedling weight.

Dry matter content of seedlings (Fig. 8).—The seedlings were dried at 105° C and weighed. The weight expressed in per cent of green weight is the dry matter content. In Fig. 8 the dry matter content of the control seedlings and those exposed to the highest intensities of radiation have been computed. The slight trend of increasing dry matter content at rising dosage is most obvious for the seed subjected to 0 per cent air humidity, statistically, however, the differences are not very pronounced.

Mortality of seedlings.—The seedlings that succumbed during the course of the germination period were recorded. Table 1 shows that mortality was highest for seedlings from seed subjected to 0 per cent air humidity.

Table 1. Mortality of seedlings (%)

Humidity \ r	0	100	200	300	600	1200	2400
0 %	0	0	0	0	16	100	—
40 %	0	1	2	2	2	1	27
100 %	1	1	0	2	1	3	32

Table 2. Condition of ungerminated seeds

Humidity \ r		0	100	200	300	600	1200	2400
0 %	a	1	2	1	1	3	55	85
	b	—	—	—	—	5	37	15
40 %	a	2	1	—	—	1	1	13
	b	—	—	—	—	1	—	12
100 %	a	1	—	1	—	2	3	11
	b	1	—	—	—	—	—	7

After the termination of the experiment all non-germinated seeds were dug up and classed.

Explanation:

a. Number of decomposed rotten seeds.
b. Number of fresh seeds and seeds showing initial germination

Condition of ungerminated seeds (Table 2).—After the termination of the experiment the condition of ungerminated seeds was investigated. The seeds were uncovered and dissected and the number of decayed and sound seeds was determined. Also in this respect it appeared that seed subjected to 0 per cent air humidity had a frequency of decayed seeds exceeding by far that of seed subjected to high air humidity.

Discussion

The effect of ionizing radiation on forest seed has previously been investigated but little. Experiments carried out in this field have mainly two objectives: induction of artificial mutations and investigation of the physiological effects of radiation.

Among the former category of experiments are found those of TOYAMA (1954) who claimed to have induced new mutations in the first generation after X-ray treatment of seed from *Pinus densiflora, Pinus Thunbergii, Cryptomeria japonica* and *Chamaecyparis obtusa.* By exposing seed of birch *(Betula pubescens)* SCHOLZ (1957) obtained, in the first generation, chlorophyll and branching variants, "mutations".

The stimulative or depressive effect of ionizing radiation on the germinability of forest seed has been studied by BALDWIN (1936) in an experiment embracing *Pinus strobus, Pinus silvestris, Picea rubra* and *Picea excelsa.* A slight retardation of the rate of germination was observed for the three latter species when exposing seed to X-rays. However, BALDWIN used one dosage only for all the species involved in the experiment. Thus, nothing is known as to the behaviour of the species at other rates of radiation. The effect of X-ray exposure on seed of Scots pine, spruce and alder was investigated by SIMAK and GUSTAFSSON (1953). In their experiments a weak insignificant effect was observed when using low dosages and an obviously depressive effect at high dosages. Simultaneously, it appeared that alder seed tolerates considerably higher dosages than seed of the conifers mentioned. SPARROW and GUNCKEL (1956) have published a list of species investigated with regards to the chronical effects of γ-rays. The list includes eleven tree and shrub species. The investigation showed clearly that the tolerances of radiation are different for various species.

The specific ionizing effects of various kinds of radiation, X-rays, γ-rays, protons, neutrons etc. manifest themselves in different ways with regard to the physiological reaction of the objects exposed. A similar result appeared in the experiments of this paper with respect to the germinability of pine seed exposed to X-rays versus γ-rays. Generally, at equal dosage, γ-rays had a less depressive effect than X-rays. The reason, which in part is probably associated with differences in ionizing density, will not be discussed further here (cf. L. EHRENBERG and NYBOM 1954).

In this study the first object was to determine for pine the reaction of seed of different ripeness to various intensities of radiation. The development of embryo, *i.e.* the length of embryo in relation to the length of embryo cavity, is a criterion on the ripeness of seed; the longer the embryo the more ripened is the seed (Simak and Gustafsson 1954). An exact determination was possible for various types of seed by means of X-ray photography.

The germination results show clearly that unripened seed (embryo class II) is much more sensitive to radiation than ripened seed (embryo class IV). Ripened seed tolerates 2—3 times more radiation than unripened seed.

Sarić (1957) who exposed seed of spring oats to X-rays found that ontogenetically young seeds are more radiosensitive than older ones. He also observed a weak stimulative effect on the development of seedlings at low rates of radiation.

In our material the stimulative effect induced by radiation is not very noteworthy from a practical point of view and it may even be dubious if analysed critically.

In the second part of the experiment spruce seed of different moisture content was exposed to various intensities of X-radiation after equilibration. It appeared evident that seed of low moisture content is more sensitive to X-rays than seed of high moisture level. Germinability of dry seed (equilibrated at 0 per cent relative air humidity) was reduced by 90 per cent after a radiation exposure of 1 200 r, whereas seed of high moisture content (equilibrated at 40 or 100 per cent air humidity, respectively) showed a germination equal to that of the control seed at the same dose of radiation. Also with respect to the development of seedlings (height and weight), seed of high moisture content showed greater tolerance to radiation than seed of low moisture level. The relationship between radiation effect and moisture content of seed has been thoroughly investigated by Caldecott (1956), L. Ehrenberg (1955 a, b), with respect to agricultural seed material. According to Ehrenberg *et al.* (1957) the effects of X-irradiation at low moisture content depends on the sum of ionizations and excitations. At high moisture content of the seed the influence of excitation is blocked and the ionizations only are effective.

By and large our experiments in spruce have produced results that agree with those of experiments in agricultural seeds mentioned above. In contrast to observations made by Ehrenberg and v. Wettstein (1955) in experiments with barley, dry matter content of the seedlings was not conspicuously increased at high radiation dosage. However, it must be pointed out that not only were the objects of investigation of a different nature—spruce and barley—but also the techniques of the experiments were different, *e.g.* with regard to the age of seedlings investigated.

The differences in the mortality of spruce seedlings grown from seeds exposed to various intensities of radiation are striking. Not only did exposed dry seeds germinate poorly, but also the seedlings exhibited such a low vigour that none of the seedlings emerging after 1 200 r survived longer than 30 days. During the same length of time but two per cent of the seedlings in the control series succumbed. The reason for the mortality of seedlings developed from dry seed seems to be that the roots developed poorly, or not at all. The roots were short and stubby and probably not capable of functioning. For this reason, the seedlings lived as long as they were able to draw upon the endosperm reserves. After termination of the germination test the ungerminated seeds were uncovered and investigated by cutting. It appeared that 85 per cent of the dry seeds exposed to a radiation of 2 400 r were decayed and but 15 per cent were still sound. The conclusion may be drawn that most of the seeds in this treatment were really killed by the radiation. At any rate, initial germination was not observed in the decayed seeds, except in a few cases.

On material of soy-beans ZACHARIAS (1956) observed a characteristic mosaic of light spots in the normal green colour of X-irradiated seedlings. This was noticed also for spruce seedlings grown from seed exposed to a high rate of radiation. In some cases the seedlings were all dark green, thereby differing clearly from normal green ones.

Comparing the results of a previous investigation on spruce and pine seed (SIMAK and GUSTAFSSON 1953) with those now presented certain differences exist. In the previous investigation, with the germinability after 30 days reduced by 50 per cent, the dosage for pine was about 900 r and for spruce about 800 r. In this experiment a germination loss of 50 per cent was observed after 50 days following a radiation dosage of 1 500 r for pine and about 3 000 r for spruce. The material and the conditions were different causing dissimilar results. Some of the factors at variance will be mentioned here with the 1953 contra 1958 differences within parenthesis: Length of germination (30 contra 50 days), moisture content (equilibrium with room temperature humidity contra equilibrium with 40 per cent relative air humidity), radiation conditions (differences of time, kV, mA, focus and filter), waiting time before sowing (in the first experiment the seeds were exposed in the city of Lund and sent by mail to Stockholm, in the second experiment the seeds were sown immediately upon irradiation), seed ripeness (seed of all embryo classes contra seed of embryo class IV only), seed provenance (pine: province of Västmanland contra province of Lapland; spruce: province of Uppland contra province of Gotland), and other data unknown *e.g.* temperature during radiation, light conditions at germination etc.

The factors mentioned play a decisive role for the result of radiation. The importance of moisture content and degree of ripeness at the time of radiation has been elucidated in this study. Remaining factors have been investigated by other research workers, *e.g.* the importance of seed size has been investigated by FRÖIER and GUSTAFSSON (1944). In another work the latter author (1947) stated that irradiated seed loses its vigour very soon after treatment. The irradiation effect also depends on the age of the seed (GUSTAFSSON 1937 a, NILAN and GUNTHARDT 1956). NYBOM *et al.* (1952) found differences in the radiation effects when the material was exposed at various temperatures. Moreover, the sensitivity of seed to ionizing radiation may depend on the provenance (TRALAU 1957) etc.

The examples cited show the importance of an accurate description of the exposed material and the radiation conditions for correct conclusions (CALDECOTT 1956). Negligence in this respect may render difficult a description of suitable dosages of radiation in experiments with other species.

The results may be *summarized* as follows:

A. X- and γ-radiation of seed of embryo classes II—IV (75 r, 150 r, 300 r, 600 r, 1 200 r and 2 400 r).

1. At low dosages X- and γ-radiations have no significant stimulative effect on the germinability of pine seed.
2. At dosages higher than 150—300 r a depressive effect appears with respect to germinability and rate of germination; sooner after X-radiation than after γ-radiation.
3. Seed of various embryo classes react differently to X- and γ-rays. At increasing dosage seed of low embryo class loses its germinative capacity faster than seed of high embryo class. However, a tendency was observed that radiation, especially γ-rays, delays the germination of high embryo class seed more than that of low embryo class seed.
4. The experiment has clearly shown that the effect of radiation depends on the ontogenetical condition of the material.

B. X-radiation of seed containing 2.8 per cent, 5.9 per cent and 25.4 per cent moisture (dosages: 100 r, 200 r, 300 r, 600 r, 1 200 r and 2 400 r).

1. X-radiation of spruce seed at various levels of moisture content had no stimulative effect on the germinability, rate of germination, number of cotelydons developed, hypocotyl length or weight of sound seedlings (and dry matter content).
2. Depressive effects on the characteristics mentioned appear at dosages between 100 r and 600 r. Seed of low moisture content (equilibrated at 0 per cent relative air humidity) is more sensitive to ionizing radiation than seed of high moisture content (equilibrated at 40 or 100 per cent air humidity).
3. The experiment shows clearly that the moisture content of seed at the time of radiation exposure is a factor of great importance for the effect of radiation in conifer seed.

Sammanfattning

Röntgen- och gammastrålningens inverkan på barrträdsfrö

I detta arbete har följande problem undersökts.

1. Röntgen- och gammastrålningens inverkan på tallfrö av olika mogenhetsgrad.
2. Röntgenstrålningens inverkan på granfrö, som bestrålats vid olika vattenhalter.

Röntgenbehandlingen utfördes med hjälp av genetiska avdelningens röntgenapparatur. För gammabehandlingen utnyttjades det s. k. gammaaggregatet på genetiska avdelningens försöksfält vid Bogesund, med radioaktivt kobolt (^{60}Co) som strålkälla (jfr planscherna 2 och 3). Embryots utveckling tjänstgjorde i detta arbete som kriterium på tallfröets mognadsgrad; ju längre den relativa embryolängden desto mognare är fröet i fråga. Bestämning av de olika frötyperna kan ske exakt med hjälp av röntgenfotografisk metodik (jfr plansch 1).

Av groningsresultatet framgår tydligt att frön av låg embryoklass förlorar sin grobarhet vid tilltagande röntgendos betydligt snabbare än frön av hög embryoklass. Nedsättningen av grobarhet och groningshastighet framträder mellan 150—300 r; vid röntgenbestrålning lite tidigare än vid gammabestrålning. Någon stimulativ verkan vid låga bestrålningsdoser har ej observerats vid vare sig röntgen- eller gammabehandling. Experimenten har entydigt ådagalagt betydelsen av materialets mognadstillstånd för strålningskänsligheten.

I den andra försöksserien röntgenbestrålades granfrö (endast embryoklass IV) av olika vattenhalt (2,8 %, 5,9 % och 25,4 %). Den önskade vattenhalten i fröna erhölls genom s. k. ekvilibrering. Bestrålning utövade ingen stimulativ verkan på grobarheten, groningshastigheten, antalet utvecklade hjärtblad, hypokotyllängden, vikten av friska plantor och torrsubstansen. Däremot iakttogs vid höga doser en starkt depressiv effekt i fråga om nämnda egenskaper (exklusive antalet hjärtblad). Depressionen inträder i fråga om de olika egenskaperna vid olika röntgendoser, i samtliga fall likväl mellan 100 och 600 r. Frön med låg vattenhalt är alltid känsligare för joniserande strålning än frön med hög vattenhalt (jfr fig. 3—8). Experimenten visade tydligt, att fröets vattenhalt är en avgörande faktor för strålkänsligheten.

Vid genetiska avdelningen har även den joniserande strålningens verkan på skogsträdsarter ingående studerats. På gammafältet vid Röskär bestrålas i olika syften tall-, gran- och poppelmaterial. Det har bl. a. visat sig, att poppelvarieteter tål högre röntgendoser än barrträdsarter. Detta beror troligen på att de stora barrträdskromosomerna i högre grad än de små poppelkromosomerna påverkas genom bestrålningen.

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Appendix

Acute irradiations of living plants of pine and poplars were carried out in the cobalt-60 field in 1957. In pine three-year old graftings of four selected plus trees (AC-1017, AC-1062, W-3003 and U-2008) were irradiated with doses from 2 000 up to 19 000 r-units. Dosages of 4 000 r-units and above were fully lethal to the graftings, whereas a dosage of 2 000 r gave plants which succumbed first a year after the treatment. All control plants, treated in a similar way without irradiation, survived and have developed normally, giving good scion material.

In poplars three varieties were used: *Populus robusta, serotina* and a variety, No. 297. They were obtained in 1956 as cut unrooted material from dr VAN VLOTEN, Wageningen, Holland. The cuttings, rooted in the spring of 1957, were irradiated in large pots during the subsequent vegetation period. Dosages ranged from 500 up to 200 000 r. No immediate effects were seen even with the highest dosages applied. Cuttings, developed in 1958 from material of the treated individuals, were obtained with 8 000 r-units or less. The stem bases of the highly irradiated individuals were still alive, however, one year after the treatment, although they were not able to develop new buds and branches with dosages above 50 000 r.

The controls grew normally. We can safely presume, therefore, that cuttings of poplars stand definitely higher irradiations than graftings of pine (and spruce, which have been treated in a similar fashion in 1958). This can be put in relation to differences in chromosome size at metaphase, as was pointed out previously for numerous angiosperms by SPARROW and CHRISTENSEN 1953, as well as by NYBOM in 1956, pine and spruce having much larger metaphase chromosomes than poplars.

Our chief interest in irradiating conifers and poplars is to induce somatic mutations. For such a purpose poplars form an ideal material, since they are easily propagated by cuttings. But conifers are from certain points of view suitable material, since they can be propagated vegetatively; in their turn by means of grafting. Similar experiments, which will be described in detail in later publications, have been carried out in other hardwood species, *e.g.* alder, *Alnus glutinosa* and *incana,* with special objects in mind. The acute dosages, suitable for the treatment of pine graftings during the vegetation period, should not exceed 2 000 r, rather lie between 500 and 1 500 r, whereas poplar cuttings should be treated with irradiations between 5 000 up to 30 000 r, the results varying with the season, as well as with the strength and healthiness of the irradiated graftings and cuttings.

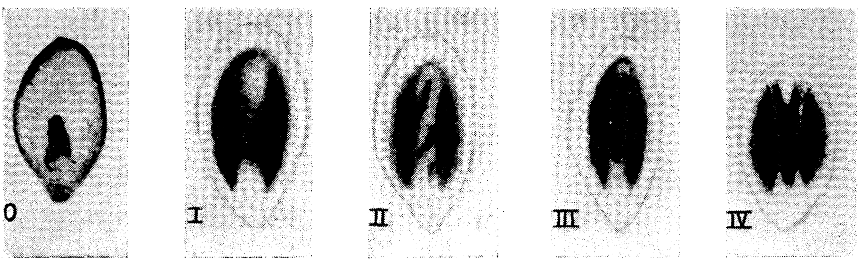
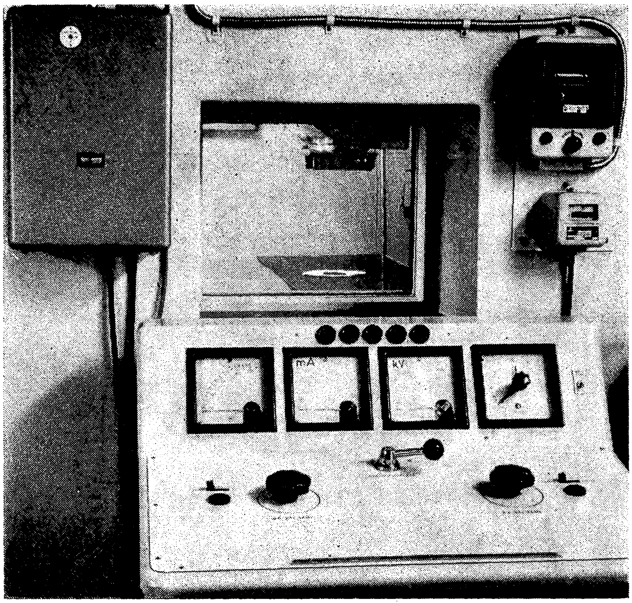


Plate 1. Above: unit used for X-ray photography of seed. The X-ray tube produces soft X-rays, so called Grenz-rays. Below: X-ray pictures of pine seed belonging to various embryo classes, 0—IV. Further details in the text.



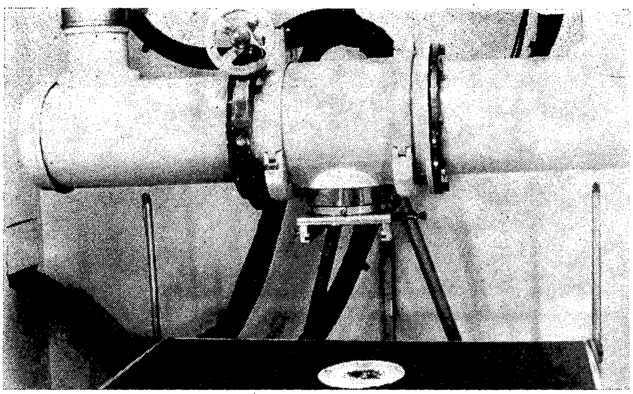
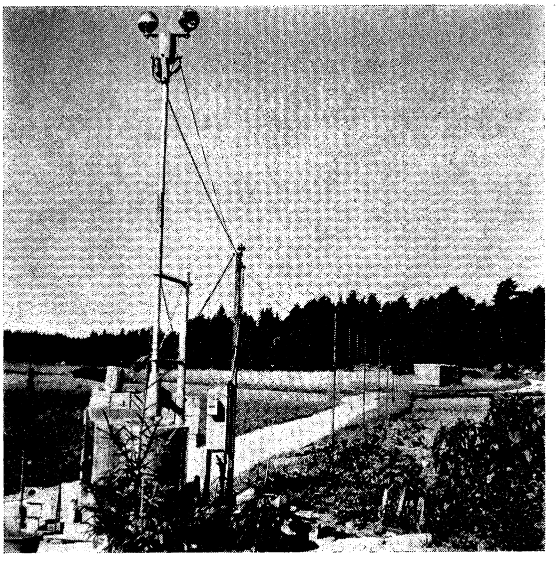


Plate 2. Unit used for X-ray exposure of forest seed. In the foreground the operator's table, on the wall the automatic dosimeter equipment. The X-ray tube is visible through the lead pane. Below: Detail picture of the tube producing hard X-rays used, *inter alia*, for mutation research.



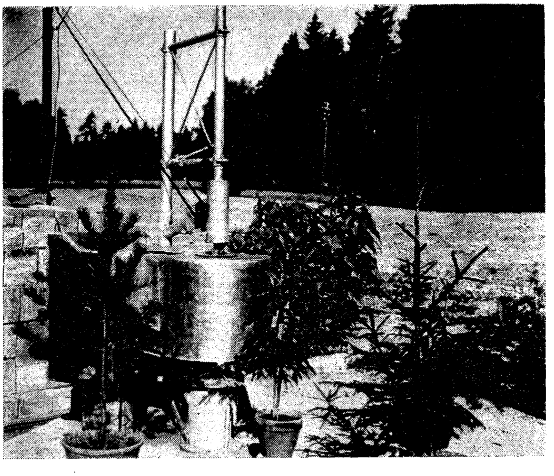
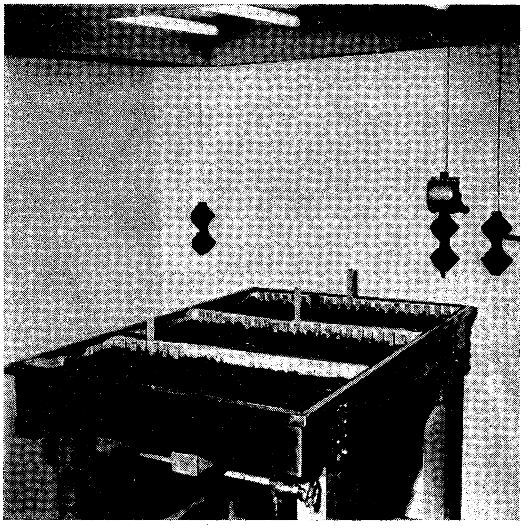


Plate 3. Gamma-unit with cobalt-60 as radioactive source. Detail picture showing the unit and the adjacent graftings of forest trees under exposure to γ-rays. At exposure the radioactive cobalt is elevated 0,75 m above the ground. When irradiation is discontinued, the cobalt is lowered into a chamber of heavy concrete blocks located 2 m below the ground surface. The unit is controlled from a place outside a fence preventing trespassing into the experimental area.



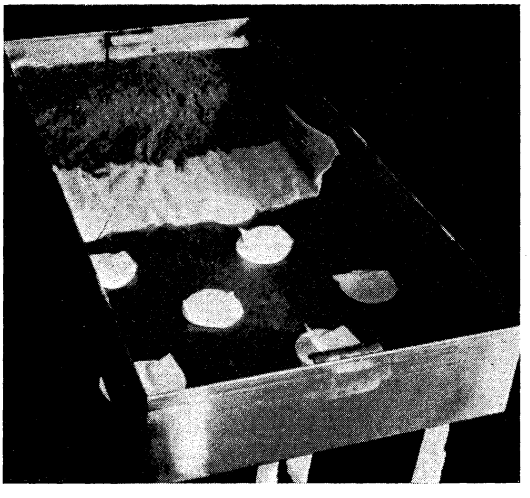


Plate 4. Arrangement for germinability tests in sand. In a basin, containing water, three sowing crates of sand are placed. Moisture, light intensity, temperature etc. are controlled. Detail picture of a sowing crate with filter paper wicks in the bottom. Keeping the sand constantly and sufficiently moist the filter paper extracts water from the basin.