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INFLUENCE OF X-RAYS ON THE FRESH WEIGHT AND THE CHLOROPHYLL SYNTHESIS OF SPRUCE SEEDLINGS

by

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1. Introduction

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Since a few years X-rays have been used by seed control services to determine the quality and the germinability of forest tree seeds. These modern techniques have also the advantage of giving immediately a fair sample of the development of embryo and endosperm. On the other hand the Röntgen analysis is very functional to put into practice a severe and efficient seed selection by which it becomes possible in view of certain scientific purposes, to work with a specific seed type.

The so called limit-rays (50 r) are used to avoid disturbant effects on the physiological and genetical character of the embryo cells by using X-rays. It is to assume that X-ray irradiations act upon reactions on high energetical level. For this reason it was set out to examine the enduring character of some physiological changes caused by long time X-ray irradiations (0 r → 10.350 r).

2. Material and employed methods

After an imbibition time of 24 h selected seeds of Picea Abies KARST. (provenance Bodensee) were irradiated during different times. The X-ray source was a Picker X-ray apparatus with following characteristics :

KV = 85
mA = 5
distance source-material = 60 cm

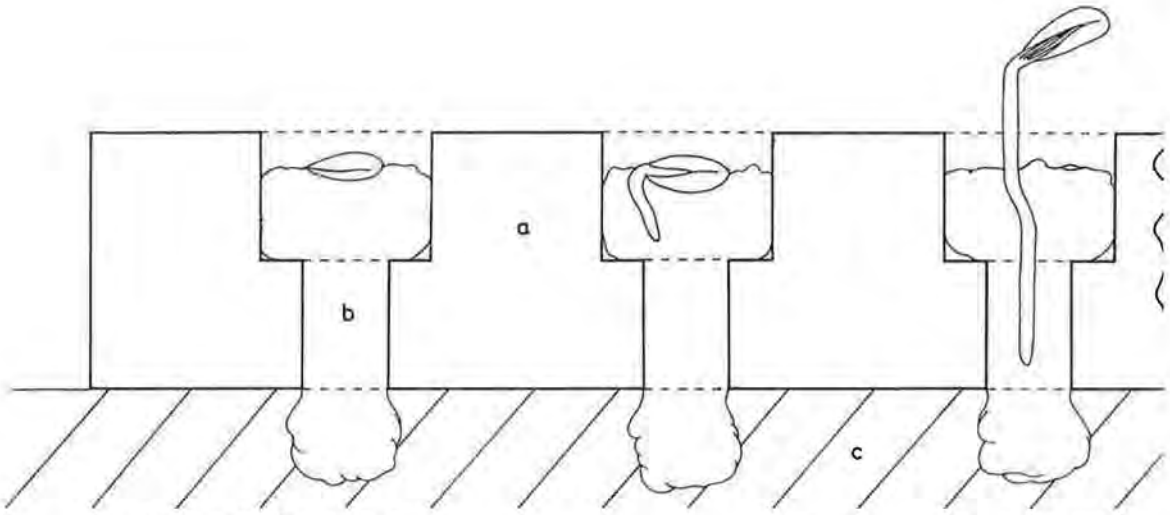

Fig. 1 : Germination method

a : support b : cotton swab
c : nutrition solution

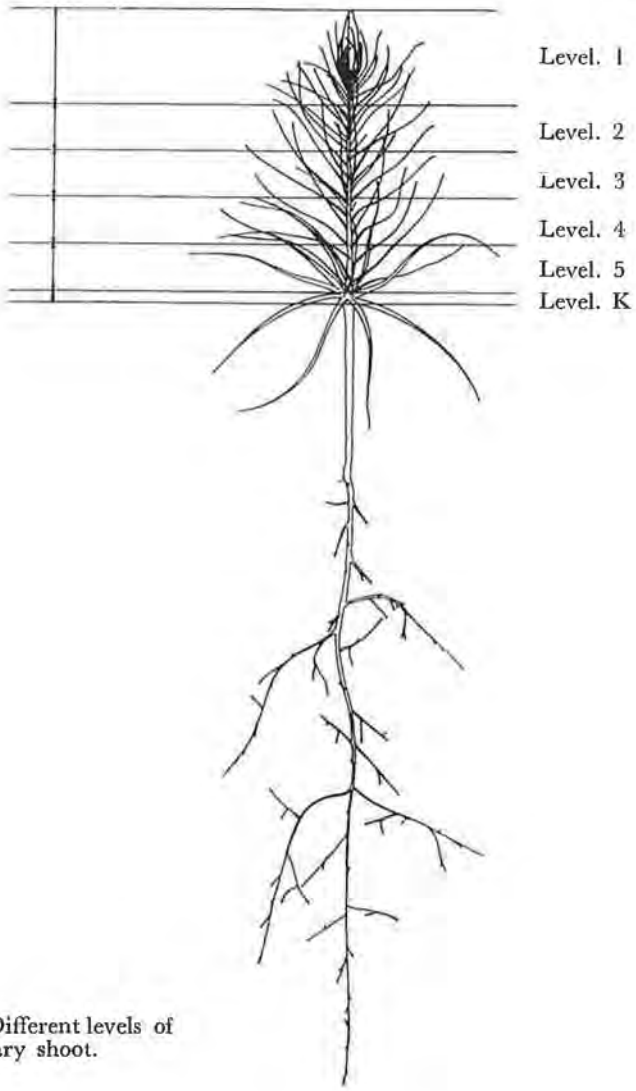

Fig : 2. Different levels of tbe primary shoot.

The irradiation times were 0 min, 8 min, 16 min, 30 min, 45 min, 60 min, and 120 min, according to a X-ray dose of 0 r, 690 r, 1380 r, 2.590 r, 3.880 r, 5.175 r and 10.350 r.

Then the irradiated seeds were put into germination conditions on cotton swabs, which were in direct contact with a nutrition.

The nutrition solution (pH = 6,7), upon which the supports for the swabs floated, contained per lit. water :

0,2 gr KNO_3 p.a.
1,15 gr ca $(NO_3)_2$. $4H_20$ p.a.
0,4 gr $MgSO_4$. $7H_20$ p.a.
0,2 gr KH_2PO_4 p.a.
0,0125 gr $FeSO_4$ p.a.

This germination method has following advantages :

a. The nutrition solution is completely screened from light by the supports so that an algae development is not possible.
b. The spreading of a seed infection is limited because each seed lies on a separate cushion.

Following conditions have been created as a growing environment for the seedlings :

Light quality : Philips TL/33.
Irradiation time : 16 h/day.
Irradiation intensity : 4.500 lux.
Temperature during the dark period : minimum 20° C.
Temperature during the light period : maximum 25° C.
Ventilation : every 2 h.
Relative air moisture : between 60 % and 80 %.

Chlorophyll contents and fresh weights of nine weeks old seedlings were determined in six levels of the developed primary shoot. As levels were accepted (fig. 2) :

a. Level K (N.K.) : Cotyledons. These are clearly to be distinguished from the other needles as a result of difference in length and thickness.
b. Level 1 (N.1) : The third part of the total crown height, taken at the top. This part contains about twelve needles.
c. Level 2 (N.2), 3 (N.3), 4 (N.4) and 5 (N.5) : The rest of the crown is divided in four equal parts.

Chlorophyll contents were determined according to the method of Mackinney (1941). Ten needles, coming from each possible combination between the considered levels of the primary shoot and the irradiation times, were crushed in a cold solvent mixture

composed of 20 vol. aq. dest. and 80 vol. of acetone p.a. After decanting and filtering the first extract through paper the needles were extracted a second and a third time with the cold solvent till the needle residue was white. The filtrate was consequently brought up to a volume of 25 ml. The extinctions were measured in a spectrophotometer Beckman type DB (1 cm type cells and narrow slit program) at 663 mμ and 645 mμ. Using following equations, the concentration of chlorophyll a, chlorophyll b and the total chlorophyll content can be calculated.

$$\log \left[\frac{I_0}{I}\right]_{663\ m\mu} = 82{,}04\ C_a + 9{,}27\ C_b$$

$$\log \left[\frac{I_0}{I}\right]_{645\ m\mu} = 16{,}75\ C_a + 45{,}60\ C_b$$

C_a = concentration of chlorophyll a in mg/ml.
C_b = concentration of chlorophyll b in mg/ml.
$C_a + C_b$ = total chlorophyll content in mg/ml.

The determinations of the fresh weight occured immediately after dividing the primary shoot in the considered levels.

3. Results

3.1. *Influence of X-rays upon the fresh weight*

TABLE I

Fresh weight of the crown levels at the different irradiation times in gram (fig.3)

Irradiation time / Level	0 min	8 min	16 min	30 min	45 min	60 min	120 min
N.K.	0,1602	0,1649	0,1930	0,1937	0,1905	0,1954	0,1604
N. 1	0,1763	0,2603	0,2922	0,3041	0,3155	0,2616	0,1597
N. 2	0,0660	0,0724	0,0822	0,0834	0,0681	0,0717	0,0609
N. 3	0,0672	0,0676	0,0785	0,0872	0,0696	0,0717	0,0670
N. 4	0,0650	0,0700	0,0752	0,0841	0,0678	0,0634	0,0663
N. 5	0,0634	0,0813	0,0852	0,1054	0,0875	0,0734	0,0660

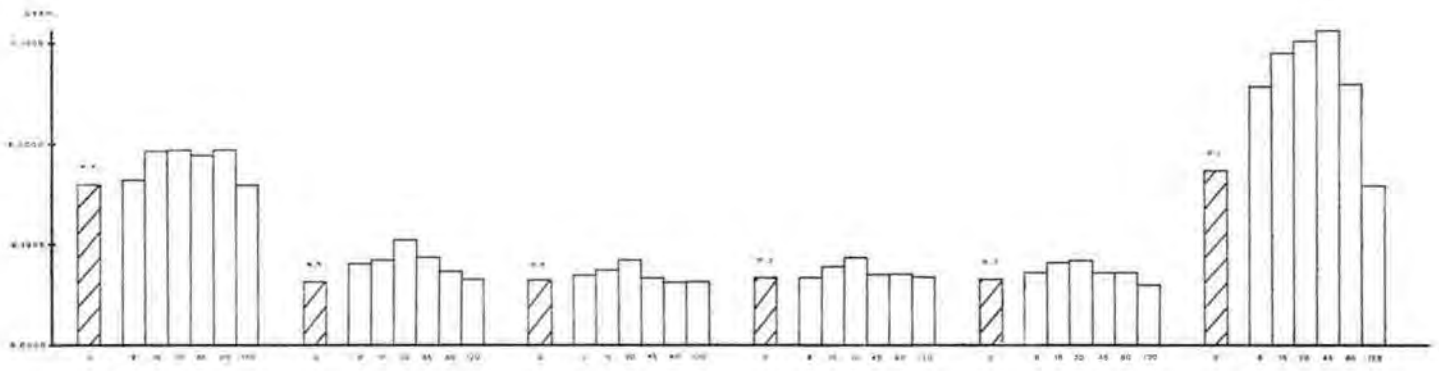

Fig 3 : Fresh weight of the crown levels at the different irradiation times in gram.

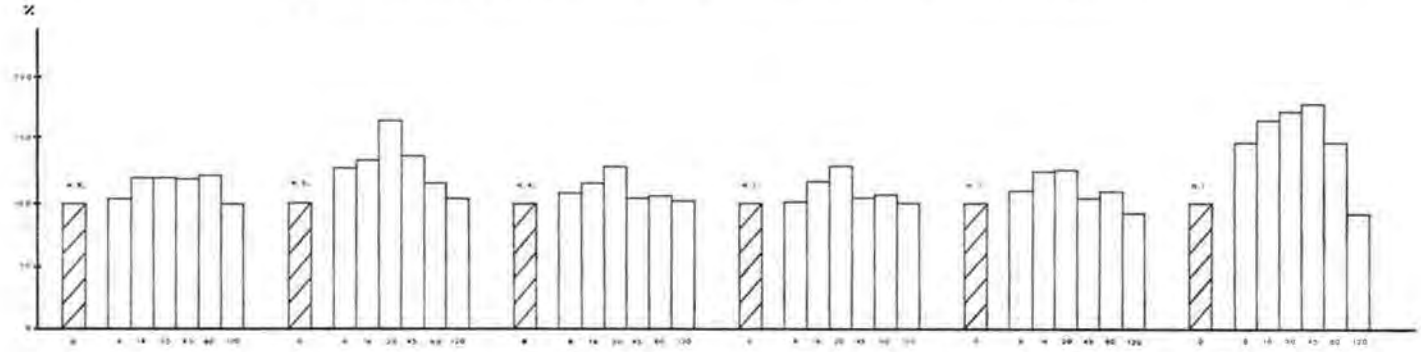

Fig 4 : Fresh weight of the crown levels at the different irradiation times in per cent of the 0 r-control.

TABLE II

Fresh weight of the crown levels at the different irradiation times in percent of the 0 r-control (fig. 4)

Irradiation time / Level	0 min	8 min	16 min	30 min	45 min	60 min	120 min
N.K.	100	103	120	121	119	122	100
N.1	100	148	166	173	179	148	91
N.2	100	110	125	126	103	109	92
N.3	100	101	117	130	104	107	100
N.4	100	108	116	129	104	106	102
N.5	100	128	134	166	138	116	104

The cotyledons (N.K.) must be considered separately.

They were already present in the seed at the irradiation moment. The rest of the needles (N.1 → N.5) didn't have such a degree of development during the X-ray treatment.

The irradiation times 0 min, 8 min and 120 min have the same influence upon the fresh weight of N.K. Concerning the other irradiation times there cannot be spoken about a real maximum, but the fresh weights are still higher (+ 20 %) than the 0 r-control. The other levels of the primary shoot (N.1, N.2, N.3, N.4 and N.5) reach a maximum at 30 min irradiation (2.590 r) and an irradiation time of 120 min corresponds with the fresh weight values for the 0 r-control.

The X-ray effect upon the fresh weight of N.1 is greater than upon the other levels. This small influence of X-rays upon N.2, N.3, N.4 and N.5 may be due to the fact that the needles have become older and it consequently brings on a fading away of the irradiation effect. The fresh weight of N.1 undergoes a great influence because the needles are just separated from the irradiated growing point and this level still contains a great part of meristematic tissue. About levels N.2, N.3, N.4 and N.5 we can notice that level N.5 undergoes the greatest irradiation influence :

N.2 (30 min) = 126 %
N.3 (30 min) = 130 %
N.4 (30 min) = 129 %
N.5 (30 min) = 166 %

The X-ray effect disappears faster by needles belonging to just arisen levels. Consequently we can suppose that the influence upon N.1 will disappear rather quickly. It is possible to come later to a situation where, for every irradiation time, the fresh weight of a certain level is identical with the fresh weight of the 0 r-control.

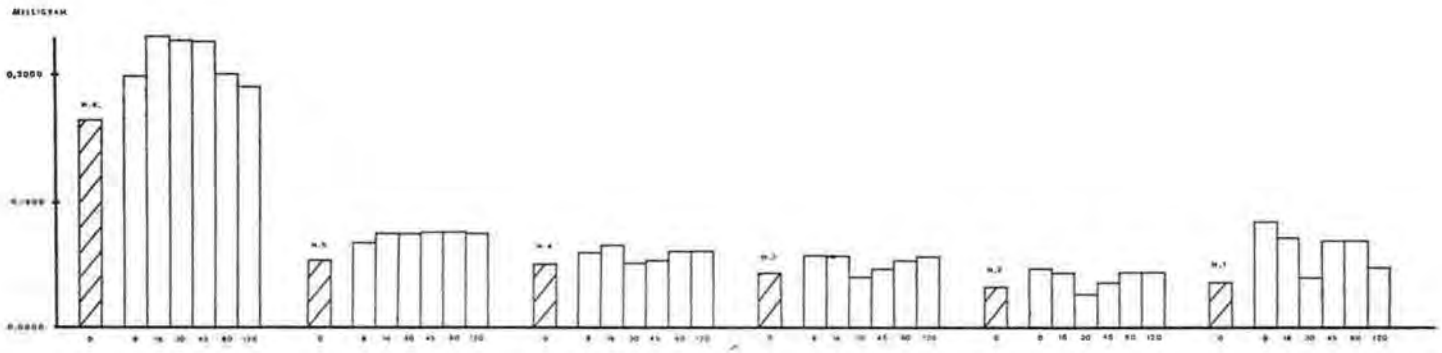

Fig 5 : Chlorophyll a content of the crown levels at the different irradiation times in miligram.

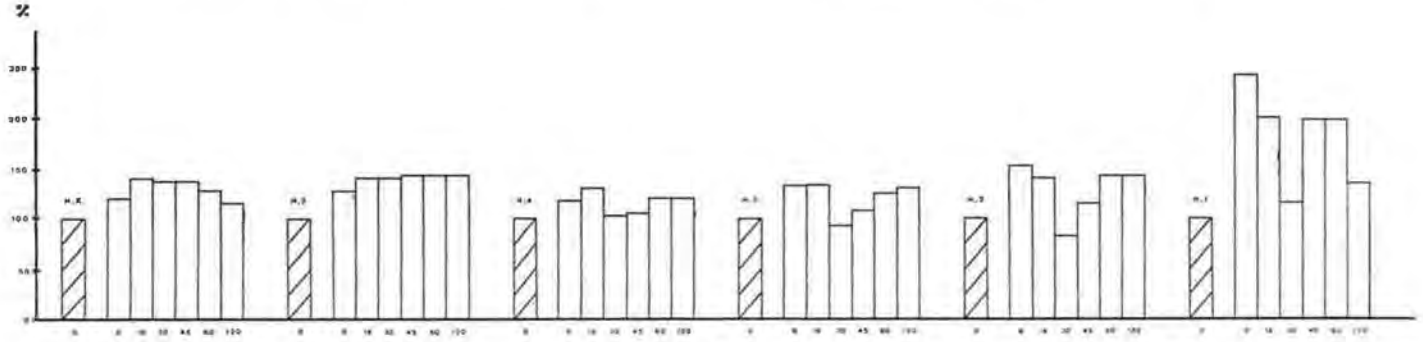

Fig 6 : Chlorophyll a content of the crown levels at the different irradiation times in per cent of the 0 r-control.

3.2. *Influence of X-rays on the chlorophyll a content, the chlorophyll b content and the total chlorophyll content*

TABLE III

Chlorophyll a content of the crown levels at the different irradiation times in milligram (fig. 5)

Irradiation time / Level	0 min	8 min	16 min	30 min	45 min	60 min	120 min
N.K.	0,16523	0,19915	0,23255	0,22860	0,22755	0,20940	0,19175
N.1	0,03443	0,08335	0,06985	0,03938	0,06793	0,06775	0,04628
N.2	0,02990	0,04548	0,04255	0,02483	0,03453	0,04240	0,04228
N.3	0,04225	0,05633	0,05645	0,03938	0,04503	0,05263	0,05520
N.4	0,04915	0,05785	0,06408	0,04990	0,05168	0,05953	0,05960
N.5	0,05225	0,06648	0,07378	0,07270	0,07493	0,07443	0,07390

TABLE IV

Chlorophyll a content of the crown levels at the different irradiation times in per cent of the 0 r-control (fig. 6)

Irradiation time / Level	0 min	8 min	16 min	30 min	45 min	60 min	120 min
N.K.	100	121	141	138	138	127	116
N.1	100	242	203	114	197	197	134
N.2	100	152	141	83	115	142	141
N.3	100	133	134	93	107	125	131
N.4	100	118	130	102	105	121	121
N.5	100	127	141	139	143	143	142

TABLE V

Chlorophyll b content of the crown levels at the different irradiation times in milligram (fig. 7)

Irradiation time / Level	0 min	8 min	16 min	30 min	45 min	60 min	120 min
N.K.	0,05608	0,07160	0,07795	0,08600	0,08415	0,08315	0,07760
N.1	0,01368	0,03900	0,02643	0,01570	0,02713	0,02830	0,02325
N.2	0,01315	0,01565	0,01453	0,00953	0,01528	0,02015	0,02033
N.3	0,01738	0,02033	0,02093	0,01570	0,01965	0,02233	0,02470
N.4	0,02088	0,02313	0,02363	0,01995	0,02103	0,02743	0,02733
N.5	0,02303	0,02930	0,02938	0,02730	0,02803	0,03263	0,03348

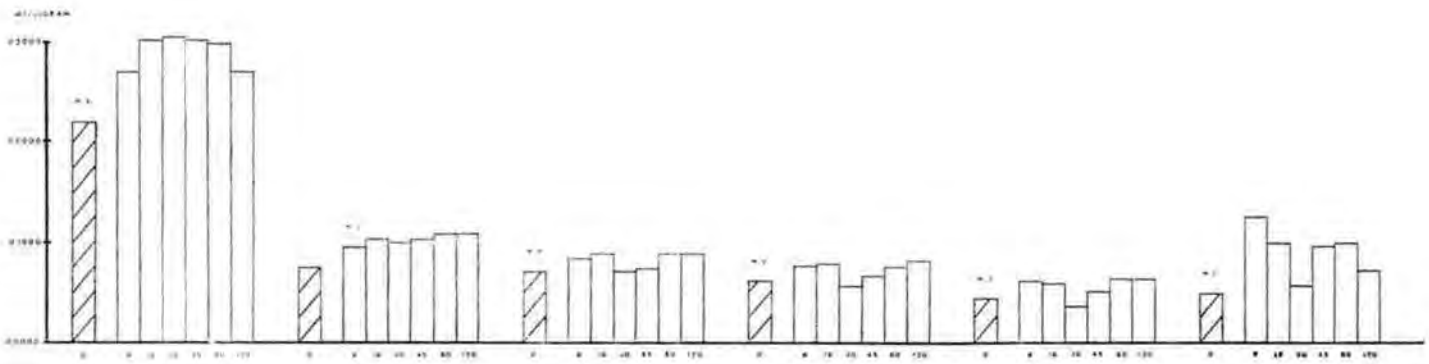

Fig 7 : Chlorophyll b content of the crown levels at the different irradiation times in milligram.

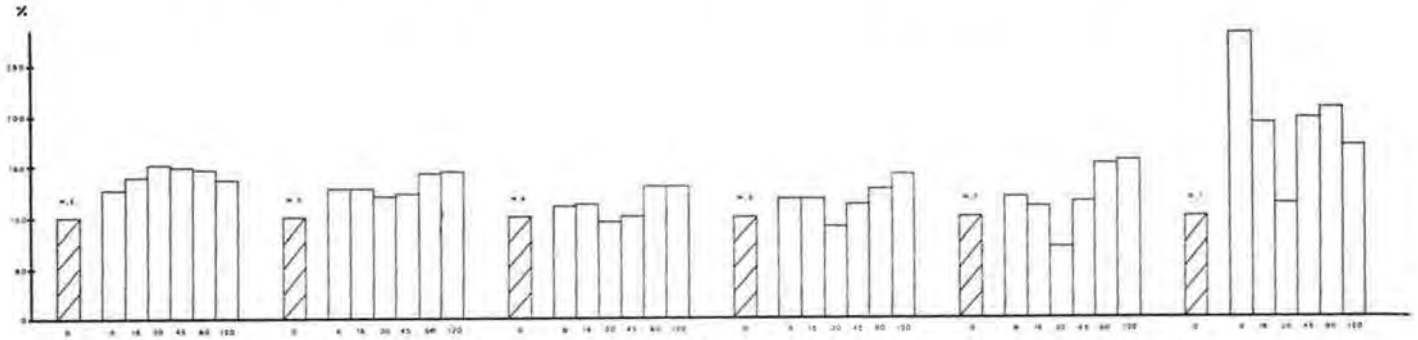

Fig 8 : Chlorophyll b content of the crown levels at the different irradiation times in per cent of the 0 r-control.

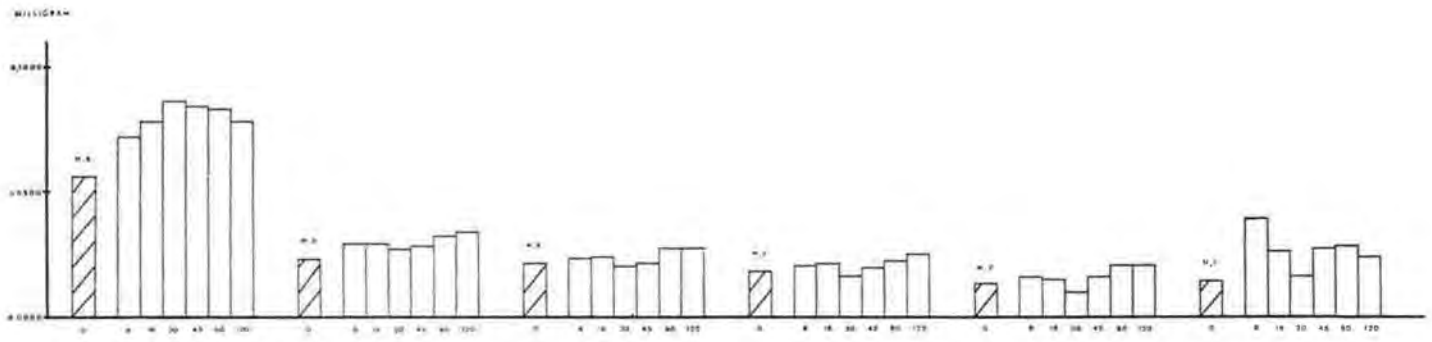

Fig 9 : Total clorophyll content of the crown levels at the different irradiation times in milligram.

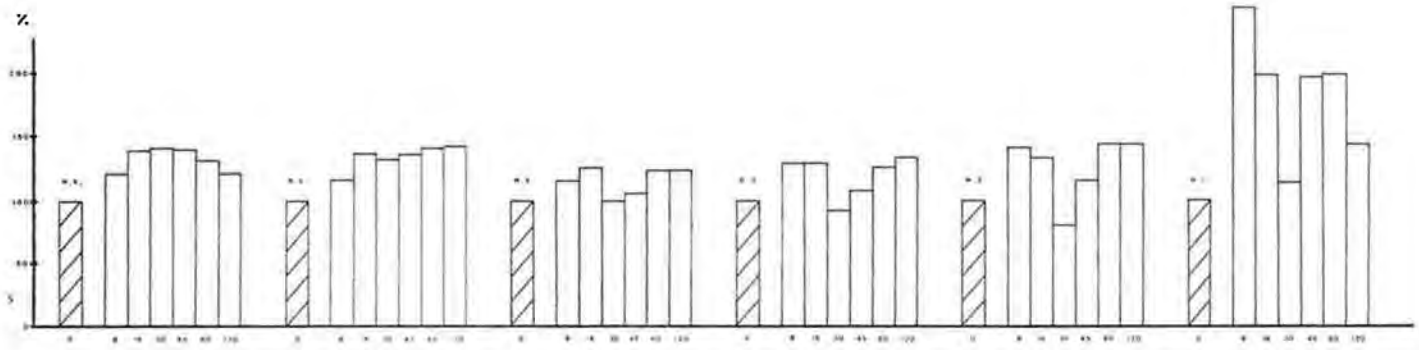

Fig 10 : Total chlorophyll content of the crown levels at the different irradation times in per cent of the 0 r-control.

TABLE VI

Chlorophyll b content of the crown levels at the different irradiation times in percent of the 0 r-control (fig. 8)

Irradiation time / Level	0 min	8 min	16 min	30 min	45 min	60 min	120 min
N.K.	100	128	139	153	150	148	138
N.1	100	285	193	114	198	207	170
N.2	100	119	110	70	116	153	155
N.3	100	117	118	90	113	128	142
N.4	100	111	113	96	101	131	131
N.5	100	127	128	119	122	142	145

TABLE VII

Total chlorophyll content of the crown levels at the different irradiation times in milligram (fig. 9)

Irradiation time / Level	0 min	8 min	16 min	30 min	45 min	60 min	120 min
N.K.	0,22131	0,27075	0,31050	0,31460	0,31170	0,29255	0,26935
N.1	0,04811	0,12235	0,09628	0,05508	0,09506	0,09605	0,06953
N.2	0,04305	0,06113	0,05708	0,03436	0,04981	0,06255	0,06261
N.3	0,05963	0,07666	0,07738	0,05508	0,06468	0,07496	0,07990
N.4	0,07003	0,08098	0,08771	0,06985	0,07271	0,08696	0,08693
N.5	0,07528	0,09578	0,10316	0,10000	0,10296	0,10706	0,10738

TABLE VIII

Total chlorophyll content of the crown levels at the different irradiation times in percent of the 0 r-control (fig. 10)

Irradiation time / Level	0 min	8 min	16 min	30 min	45 min	60 min	120 min
N.K.	100	122	140	142	141	132	122
N.1	100	254	200	114	198	200	145
N.2	100	142	133	80	116	145	145
N.3	100	129	130	92	108	126	134
N.4	100	116	125	100	104	124	124
N.5	100	127	137	133	137	142	143

For the chlorophyll a content as well as for the chlorophyll b content the same conclusions may be made and consequently the same may be said about the total chlorophyll content because these data were calculated by means of the chlorophyll a and chlorophyll b contents.

Such as for the influence of X-ray irradiations on the fresh weight the cotyledons must be considered separately : at the irradiation moment these needles were already present in the seed.

An irradiation time of 8 min has the most favourable influence on N.1 :

	in mg	in % of the 0 r-control
chl. a	0,08335	242
chl. b	0,03900	285
total chl.	0,12235	254

At longer irradiation times the chlorophyll content decreases and a minimum is attained at 30 min (probably between 30 min and 45 min), after which the content increases again. The value for 120 min in this level (N.1) is very low because a dose of 10.000 r brings on to the fact that the seedling is poor in needles or that the needles begin to die off. It is most probably that a dose of 10.000 r determines the survival limit.

This minimum at 30 min also appears in N.2, N.3 and N.4, but it is not so clearly pronounced when the needles are older (N.2 → N.3 → N.4). Further we can notice that :

a. The different irradiation times give about the same value.

b. The relative values for the 8 min irradiation time decrease faster from N.1 to N.5 than in case of the other irradiation times.

During the X-ray treatment the needles are not yet formed by the apex of the shoot with exception of the cotyledons. At this stage of the germination not many enzymatic processes have occured and we may suppose that a great part of the precursors, necessary for formation of chlorophyll, were not synthesized.

By examination of mentioned data it brings out that X-rays have an influence on the rapidity of the chlorophyll synthesis (« rapidity » effect). A certain quantity of X-rays (30 min = 2.590 r) is the cause of a slow chlorophyll synthesis. This quantity of X-rays probably has an influence on the formation reaction on high energetical level.

This « rapidity » effect gets lost, according to the fact that the needles become older (N.1 → N.5). It occurs on a rapid manner with short irradiation times (8 min, 16 min) and the fact remains

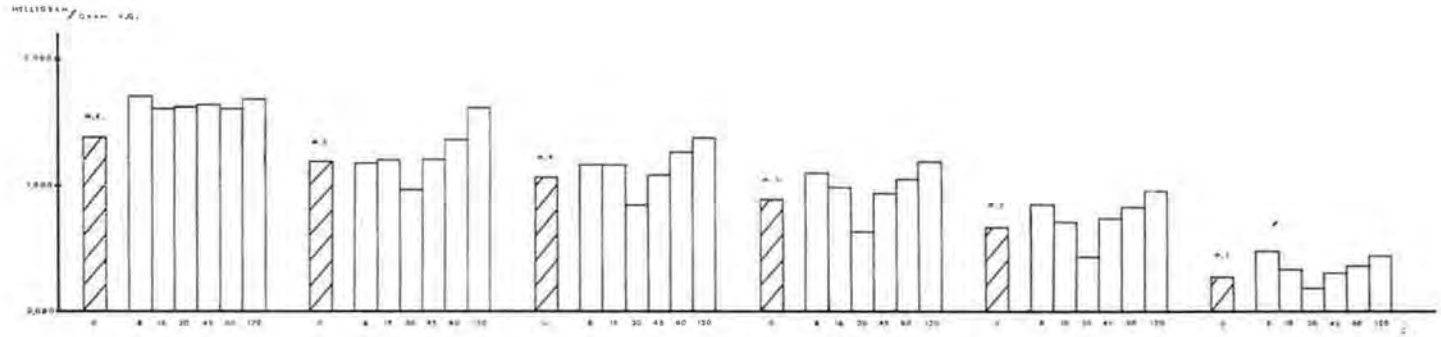

Fig 11 : Total chlorophyll content of the crown levels in mg/gr fresh weight.

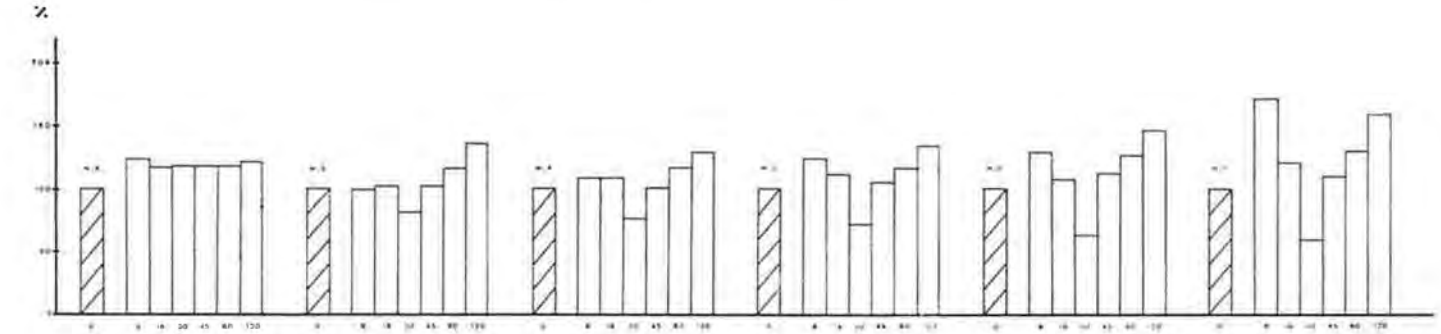

Fig 12 : Total chlorophyll content of the crown levels per gr fresh weight in per cent of the 0 r-control.

a long time with high quantities of X-rays. The disappearance of the irradiation effect was also noticed during the discussion of X-ray influences on the fresh weight.

As the cotyledons are present in the seed and that some already have a green colour during the X-ray treatment, it can be supposed that the greater part of the enzymatical and synthetical processes of the chlorophyll synthesis are already at an end. The data of this level (N.K.) correspond with the results of the influence of X-rays on the fresh weight. It can be named a « formative » effect : the chlorophyll content increases in proportion to the fresh weight.

On the other hand the supposition can be made that N.4 and N.5 are transitional stages. Here the « rapidity » effect has only a little influence and the needles, according as they become older, will have the same chlorophyll contents as the cotyledons : when the « rapidity » effect disappears the « formative » effect appears more clearly.

In the discussion of X-ray influences on the formation of chlorophyll has appeared that the conclusions for the chlorophyll a, the chlorophyll b and the total chlorophyll content are parallel. In future, only the total chlorophyll content will be considered.

TABLE IX

Total chlorophyll content of the crown levels in mg/gr fresh weight (fig. 11)

Irradiation time / Level	0 min	8 min	16 min	30 min	45 min	60 min	120 min
N.K.	1,381	1,709	1,608	1,624	1,636	1,598	1,678
N.1	0,273	0,470	0,329	0,177	0,301	0,356	0,436
N.2	0,652	0,844	0,695	0,413	0,731	0,824	0,954
N.3	0,887	1,101	0,986	0,632	0,929	1,045	1,193
N.4	1,077	1,158	1,166	0,818	1,086	1,256	1,382
N.5	1,187	1,179	1,211	0,957	1,208	1,371	1,619

TABLE X

Total chlorophyll content of the crown levels per gr fresh weight in percent of the 0 r-control (fig. 12)

Irradiation time / Level	0 min	8 min	16 min	30 min	45 min	60 min	120 min
N.K.	100	124	117	118	118	118	122
N.1	100	172	121	59	110	130	160
N.2	100	129	107	63	112	126	146
N.3	100	124	111	71	105	118	134
N.4	100	108	108	76	101	117	129
N.5	100	99	102	81	102	116	136

The fact that an irradiation time of 30 min has a positive influence upon the fresh weight and a negative effect upon the chlorophyll synthesis brings on that at the 30 min irradiation time the chlorophyll content expressed in function of the fresh weight is very low :

	in mg chl./gr F.W.	In % of the 0 r-control
N.1/30 min	0,177	59
N.2/30 min	0,413	63
N.3/30 min	0,632	71
N.4/30 min	0,818	76
N.5/30 min	0,957	81

The interaction of both the effects, « rapidity » effect and « formative » effect appears clearly :

a. Level 1 : The « rapidity » effect is more important than the « formative » effect.

b. Level 4 and level 5, 16 min and 8 min : The « rapidity » effect hardly acts a great part and is replaced by the « formative » effect.

c. Irradiation time 8 min, 16 min, 30 min and 45 min : The 0 r-control value is pursued from N.1 to N.5 (marked on table 10). Irradiation times 120 min and 60 min make an exception because the « rapidity » effect remains for a longer time.

d. Level N.K. : Both effects exert little influence on the cotyledons.

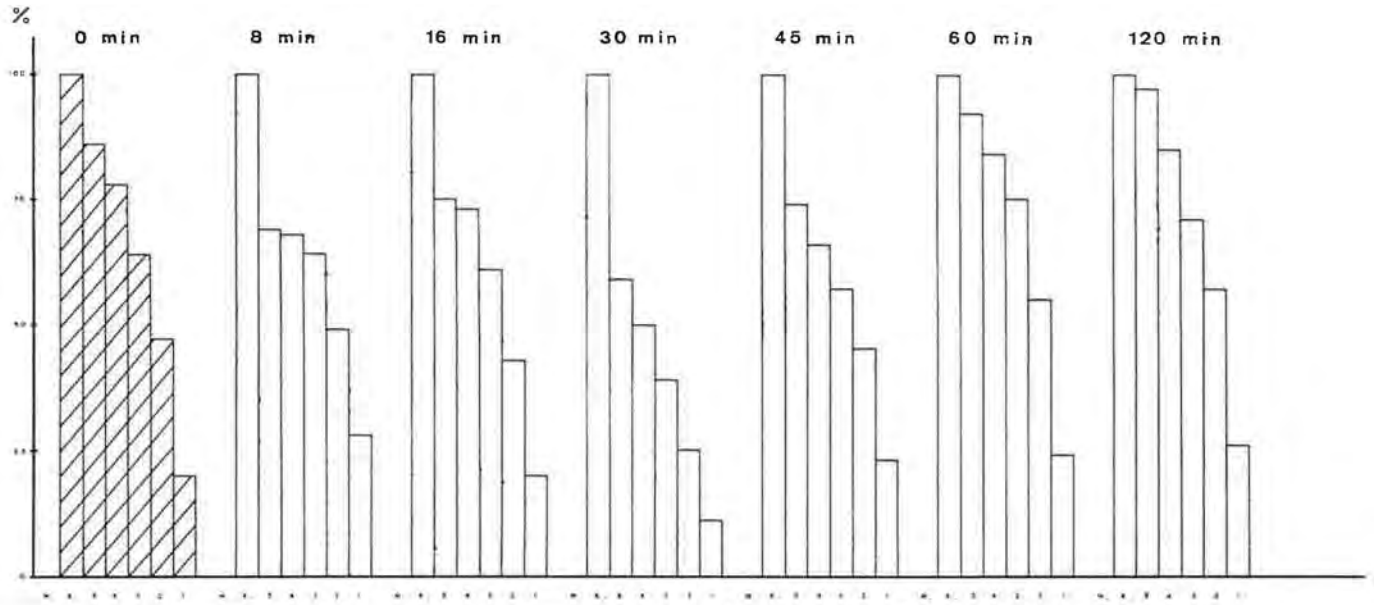

Fig 13 : Total chlorophyll content per gram fresh weight in per cent of the cotyledon-level (N.K.).

TABLE XI

Total chlorophyll content per gram fresh weight in percent of the cotyledon-level (N.K.) (fig. 13)

Level / Irradiation time	N.K.	N.5	N.4	N.3	N.2	N.1
0 min	100	86	78	64	47	20
8 min	100	**69**	**68**	64	49	28
16 min	100	**75**	73	61	43	20
30 min	100	59	50	39	25	11
45 min	100	74	66	57	45	18
60 min	100	92	84	70	55	24
120 min	100	97	85	71	57	26

The chlorophyll contents of the 0 r-control decrease regularly from N.5 to N.1. On the contrary seedlings, coming from X-ray treated seeds, don't have such a regular decrease because there is an interaction between « rapidity » and « formative » effect. Both effects are more active in N.1, N.2, N.3, N.4 and N.5 as in N.K. :

a. 60 min and 120 min : For each considered crown level the percentages are higher than the 0 r-control values, because the « rapidity » effect causes a fast chlorophyll formation.

b. 45 min and especially 30 min : Slow chlorophyll formation caused by the negative effect of X-rays on the chlorophyll synthesis.

c. 16 min : Identical to the 0 r-control, except N.4 and N.5. The « rapidity » effect has been crowd out by the « formative » effect (marked on table 11).

d. 8 min : Fast formation of chlorophyll in N.2 and N.1 and a slow formation in N.5 and N.4 caused by a rapid appearance of the « formative » effect (marked on table 11).

Summary and conclusion

Since a few years many X-ray techniques have been used for the control of forest tree seeds.

The influence of high quantities of X-rays and the enduring character of these irradiations have been examined.

After an imbibition time of 24 h selected seeds of Picea Abies KARST. (provenance Bodensee) were irradiated during different periods, corresponding with 0 r, 690 r, 1.380 r, 2.590 r, 3.880 r,

5.175 r and 10.350 r. Determinations of the fresh weight and the chlorophyll content in different levels of the primary shoot have been done on nine weeks old seedlings.

1. *Influence of X-rays on the fresh weight*

   a. An irradiation time of 30 min (2.590 r) has a positive influence on the fresh weight of each considered primary shoot level.
   b. The irradiation effect disappears according as the seedlings grow up and the slightest influence has been observed in the lowest levels of the primary shoot.
   c. The influence of the irradiation is not so strongly marked in the cotyledons because these needles are already present in the seed at the moment of the applied irradiations.

2. *Influence of X-rays on the chlorophyll synthesis*

   a. An irradiation time of 30 min (2.950 r) has a negative influence on the chlorophyll synthesis. This quantity of X-rays probably brakes high energetic reactions, necessary for the formation of chlorophyll. Irradiation times of 8 min (690 r) and 120 min (10.350 r) have a positive influence.
   b. This X-ray effect get lost according as the needles become older and is replaced by a « formation » effect. This influence is nothing else than a chlorophyll synthesis taking place in proportion to the fresh weight.
   c. The cotyledons must be considered separately. They are only liable to the « formation » effect because a great part of the precursors, necessary for the formation of chlorophyll, exists before the X-ray treatment.

The influence of the used irradiations (0 r → 10.350 r) is in the beginning of the development of the seedling very high, but disappears when the plant grows up. There can be expected that on one year old seedlings the consequences of a X-ray treatment can hardly be discovered anymore.

On the other hand there must be mentioned that an irradiation time of 120 min (10.350 r) brings on a reduction of the germination percentage. Probably 10.000 r is the lethal limit dose.

LITERATURE CITED

1. EVENARI, M., 1956. — Seed germination. *Radiation Biol. III:* 519-549.
2. GUSTAFSSON, Å. and SIMAK, M., 1958. — Effect of X- and γ-rays on Conifer Seed. *Medd. Stat. Skogforst.* Inst. Band 48/5 : 1-20.
3. GUSTAFSSON, Å. and MILAK, M., 1956. — X-ray diagnostics and seed quality in forestry. *Int. Union of For. Res. Orgn. 12th congress Oxford:* 398-413.
4. LANG, A., 1965. — Effect of some internal and external conditions on seed germination. *Handb. Pflanzenphys.* XV/2 : 848-893.
5. MACKINNEY, G., 1941. — Absorption of light by chlorophyll solutions. *Journ. Biol. Chem.* 140 : 315-322.
6. MUELLER-OLSEN, C., and SIMAK, M., 1954. — X-ray photography employed in germination analysis of Scots Pine (*Pinus silvestris L.*). *Medd. Stat. Skogsforsk.* Inst. Band 44/6 : 1-9.
7. MUELLER-OLSEN, C., MILAK, M. and GUSTAFSSON, Å., 1956. — Germination analysis by the X-ray method : Norway spruce. *Medd. Stat. Skogsfork. Inst.* Band 46/1 : 1-12.
8. SIMAK, M. and GUSTAFSSON, Å., 1953. — X-ray photography and sensitivity in forest tree species. *Medd. Stat. Skogsforsk. Inst.* Band 39/3-4 : 458-468.
9. THAS, J., 1966. — A quantitative determination of the total chlorophyll content in higher plants. *Med. Rijksfac. Landb. Gent.* XXXI, Nr. 4. : 1297-1301
10. VEGIS, A., 1961. — Samenkeimung und vegetatieve Entwicklung der Knospen. *Handb. Pflanzenphys.* Band XVI : 168-298.