PRELIMINARY REPORT ON THE EFFECT OF GAMMA-IRRADIATION ON THE GERMINATION OF SOME WESTERN CONIFEROUS SPECIES

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INTRODUCTION

Earlier experiments in this field have indicated that ionizing radiation could cause permanent genetical effects, lethal or beneficial mutations, morphological modifications, and other effects in plants. Relatively, much is known regarding the radiosensitivity of Angiosperms, but many questions concerning the effects of irradiation on Gymnosperms, particularly the Western coniferous species, remain to be answered. Data on the sensitivity of conifer seed to different kinds of rays are in some instances conflicting. The lethal dose varied depending on the species, types, range, and dose rate. It also depends on acute irradiation (Shields and Rickard, 1961; Sparrow et al., 1963) or on chronic radiation (Mergen and Stairs, 1962; Pedigo, 1963; Sparrow et al., 1961).

The seed stage is a convenient phase in the plant's life cycle for use in radioecological studies to determine relative radiosensitivity of species and the effects of various factors on radiosensitivity.

This work is primarily designed to obtain some information on the radiosensitivity of *Pseudotsuga mepziesii* (Mirb.) Franco (Douglas-fir), Picea *sitchensis* (Bong.) Carr. (Sitka spruce), and *Tsuga heterophylla* (Raf.) Sarg. (western hemlock). A secondary objective is to increase variation by induced mutations.

In the research reported here, the effects of different doses of acute γ irradiation on seed germination were studied. Possible evidence of radiostimulation of germination was also sought.

MATERIALS AND METHODS

The seeds of the following species were used in this study:

1. Douglas-fir collected from two different locations: (a) from Duncan, B.C., 1959 crop, (Coastal); (b) from Richter Pass, B.C., 1964 crop, (Interior).
2. Sitka spruce collected by Gordon Roche in 1963 from Queen Charlotte Island.
3. Western hemlock collected from Marine Drive, University of British Columbia campus in 1964.

The filled seeds were separated from the empty ones using X-ray [Softex type EM (SEM-12) X-ray unit]. Only the filled seeds were used in these experiments.

A trial experiment was carried out using γ irradiation from a Co60 radiotherapy unit at the B. C. Cancer Institute. The following doses were given: 0, 2, 5, 10, and 15 kr at a dose rate of 40 r/sec.

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For the second experiment samples containing 150 seeds were sealed in paper bags and γ irradiated using Co^{60} (220 γ cell) source. The given doses were as follows: 0 (Control), 0.5, 2, 5, and 10 kr. (The 15 kr. treatment was omitted because it gave no germination in the first experiment.) The exposure rate was 500 kr./hr., i.e., ca. 139 r/sec.

After exposure treatments, the seed lots from each group were divided into two sub-treatments: (a) sown immediately; (b) soaked in tap water for 24 hours and then stratified at 2°-3° C for fifteen days (naked stratification, Allen and Bientjes, 1958). The seeds were then sown.

Germination took place on "perelite" in Petri dishes. Seeds were incubated at 25° C in the dark.

Three replications were used in a factorial design. Germination counts were made every two days for a 42-day period for nonstratified seeds, and for a 28-day period for the stratified ones. The seed was considered germinated when the radicle was normal in appearance and exceeded the length of the seed.

RESULTS AND DISCUSSIONS

The mean values of germination percent for different species and different treatments are given in table 1.

Table 1. -- Results of germination of the three species after four treatments and the control - Germination percent.

Species		Control		500 r		2,000 r		5,000 r		10,000 r	
		St 1/	Unst 2/	St.	Unst.	St.	Unst.	St.	Unst.	St.	Unst.
Douglas-fir (Coastal)	A 3/	74.7	81.3	77.3	80.0	76.0	74.7	54.7	38.7	13.3	8.0
	B 4/	100.0	100.0	103.5	98.4	101.7	91.9	73.2	47.6	17.8	9.8
Douglas-fir (Interior)	A	80.0	78.7	82.7	57.3	90.7	72.0	86.7	50.7	49.3	10.7
	B	100.0	100.0	103.4	72.8	113.4	91.5	108.4	64.4	61.6	13.6
Sitka spruce	A	41.3	38.7	33.3	14.7	20.0	5.3	0	4.0	0	0
	B	100.0	100.0	80.6	38.0	48.4	13.7	0	10.3	0	0
Western hemlock	A	70.7	89.3	61.3	76.0	45.3	72.0	5.3	37.3	0	8.0
	B	100.0	100.0	86.7	85.1	64.1	80.6	7.5	41.8	0	9.0

1/ Stratified.
2/ Unstratified.
3/ A = Means of three replications.
4/ B = Germination as a percent of the control.

The effect of total exposure on the germination of Douglas-fir seeds (Coastal) as a percent of the control is shown in fig. 1. There was a general decrease in germination percent as the dose increased, except at 500 and 2,000 r. which stimulated the germination by 3.48% and 1.74% over the control, respectively. However, these differences were not significant at the 5% level. The stratified seeds gave high germination percent over the unstratified seeds at all levels of irradiation.

The LD50 value, (dosage at which germinability is reduced by 50% relative to the control, Snyder et al., 1961) for stratified seeds was about 7.1 kr. and for unstratified seeds about 4.8 kr. This means that the stratification increased the dose required to reduce germination by 50%.

The same trend was generally found with Douglas-fir seeds from the interior, fig. 2. Furthermore, the 500 r. and 2,000 r. doses increased the germination significantly over the control. This is the only case in which irradiation stimulated the germination. The analysis of variance showed a highly significant difference between sub-treatments, i.e., stratified and unstratified seeds. The stratification increased the germination significantly.

The germination of Sitka spruce seeds was affected severely by irradiation. The LD50 for stratified seeds is about 1.9 kr. and for unstratified seeds 0.4 kr. There was not any stimulation of germination in this species. The seeds of western hemlock showed the same response to irradiation by decreased germination as the total dose increased, figs. 3 and 4. However, the germination of the stratified seeds was significantly lower than the unstratified ones. This is the reverse of the other two species. The LD50 for stratified seeds is about 2.8 kr. and for unstratified seeds 4.3 kr., fig. 4.

Comparing figs. 1 and 2 and tables la and lb it could be noted that Douglas-fir seed from the interior location gave higher germination percentages than those from the coastal location.

Figures 5 to 12 show the rates of seed germination for the different species as affected by different doses of y irradiation. The differences were less pronounced within each species in the initial stages of germination, but became more distinct towards the end of the germination period.

The deleterious effects of y irradiation on seed germination as reported here is in agreement with most of the studies in this field. The radiosensitivity varied considerably among the species studied. The LD50 values show that Douglas-fir (coastal and interior sources) is more tolerant to y irradiation than Sitka spruce and western hemlock, table 2.

Table 2. -- LD_{50} in kr. for the three species based on germination.

Species	Stratified seeds	Unstratified seeds	Mean
Douglas-fir (Coastal)	7.1	4.8	5.95
Douglas-fir (Interior)	11.2	6.4	8.80
Sitka spruce	1.9	0.4	1.15
Western hemlock	2.8	4.3	3.55

Previous studies of the radiosensitivity of higher plants as determined by exposure to chronic irradiation have shown that radiosensitivity varies among species by as much as 500 fold.

EFFECT OF DIFFERENT DOSES OF GAMMA-IRRADIATION ON SEED GERMINATION .

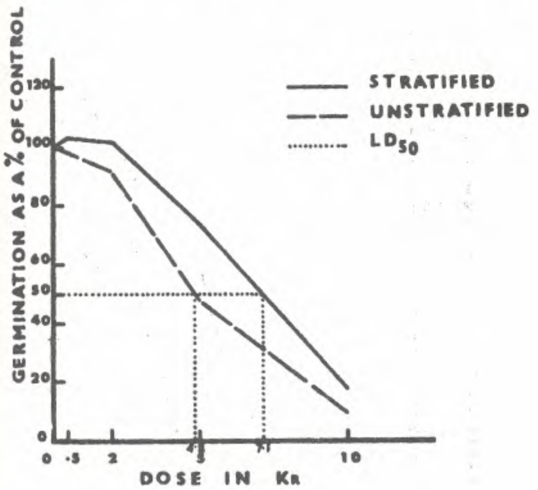


FIG. 1. **Douglas-fir (Coastal)**

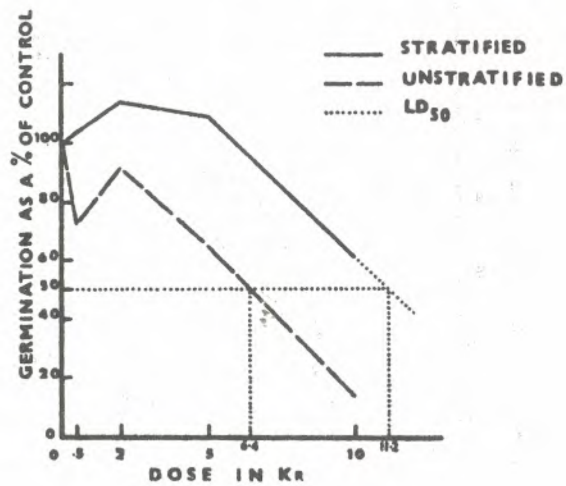


FIG. 2. **Douglas-fir (Interior)**

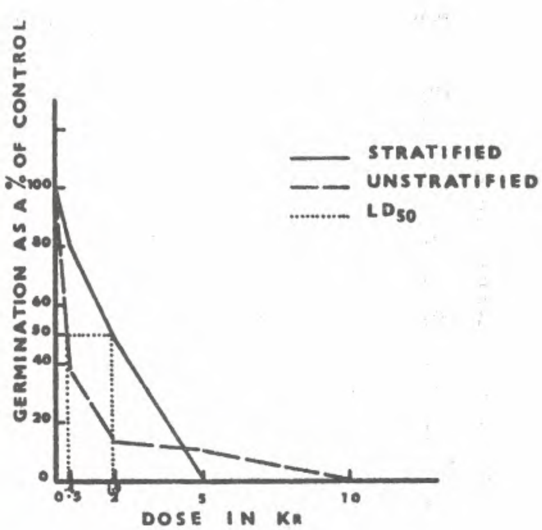


FIG. 3. **Sitka spruce**

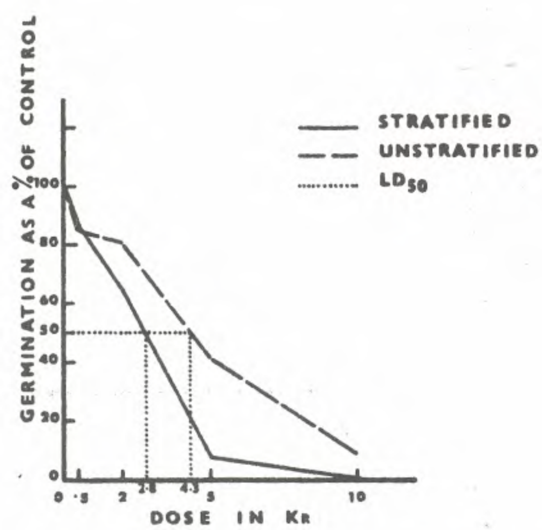
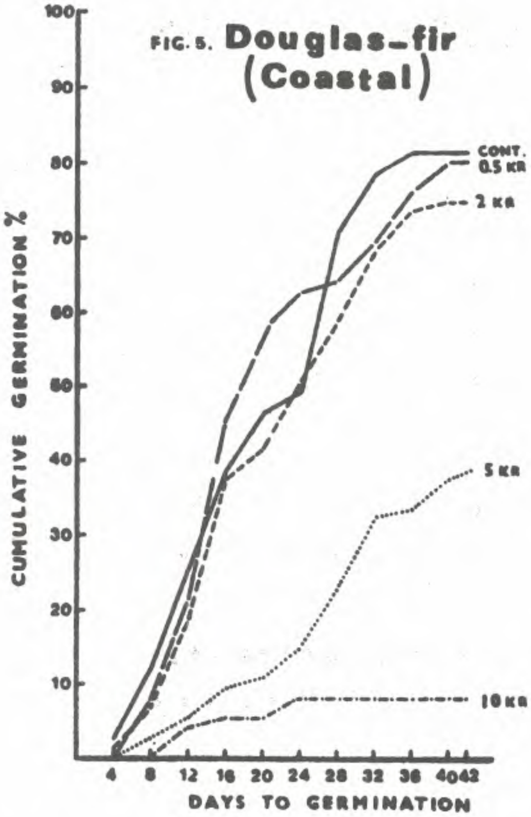
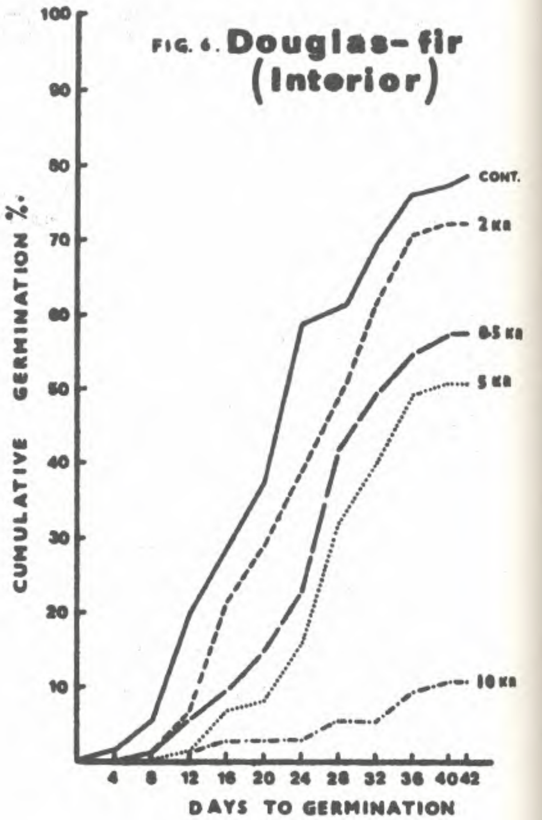
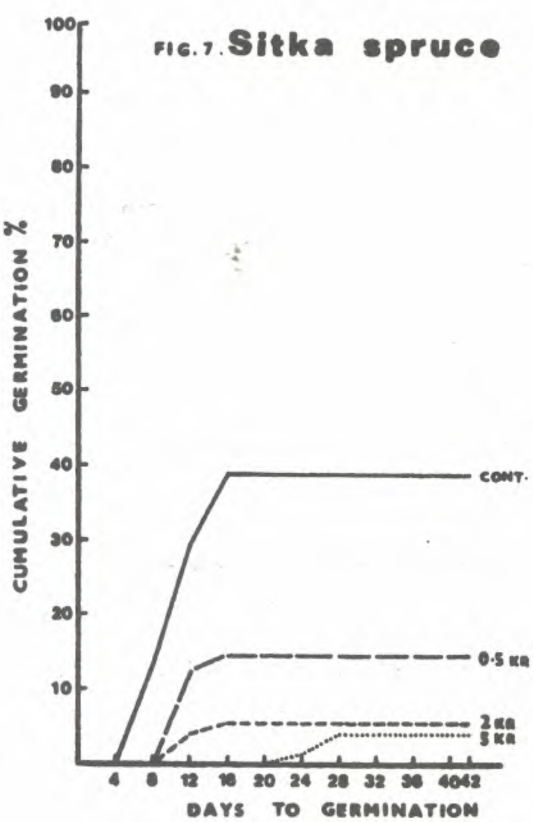


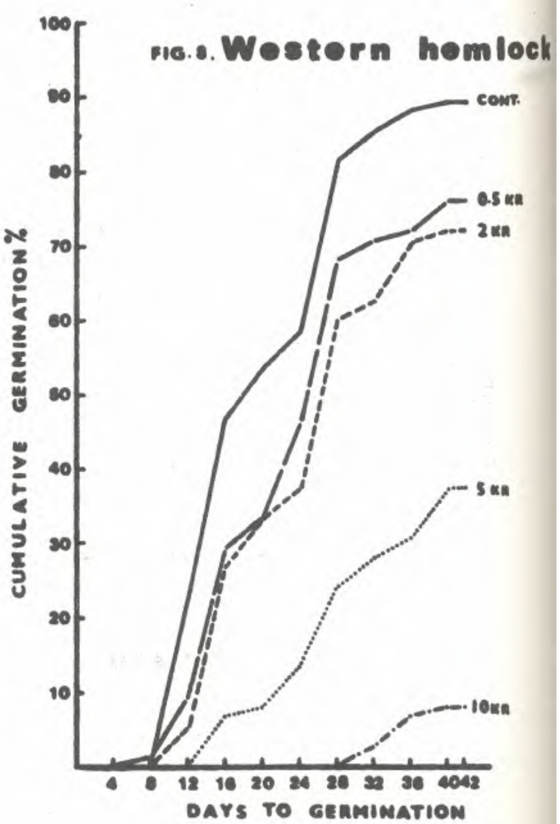
FIG. 4. **Western hemlock**

RATE OF GERMINATION OF GAMMA-IRRADIATED SEEDS (UNSTRATIFIED).









RATE OF GERMINATION OF GAMMA-IRRADIATED SEEDS (STRATIFIED).

FIG. 9. **Douglas-fir (Coastal)**

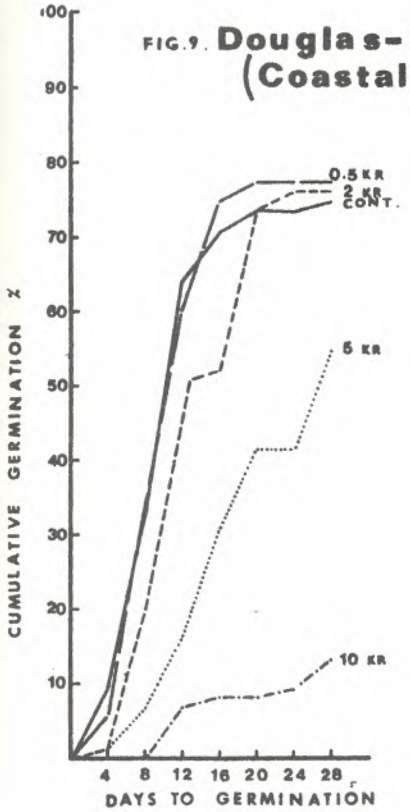


FIG. 10. **Douglas-fir (Interior)**

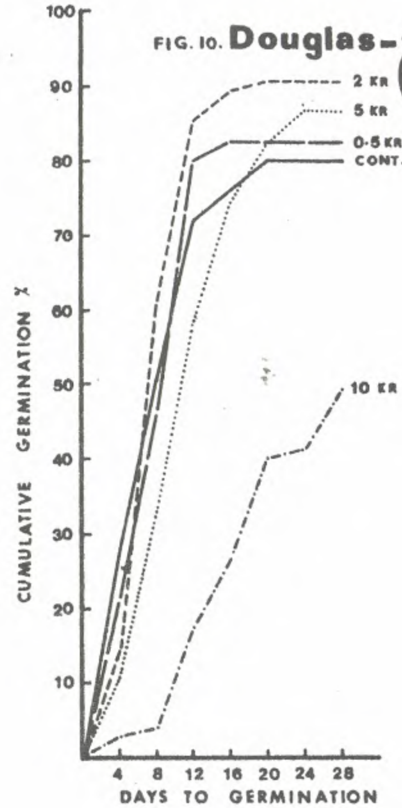


FIG. 11. **Sitka spruce**

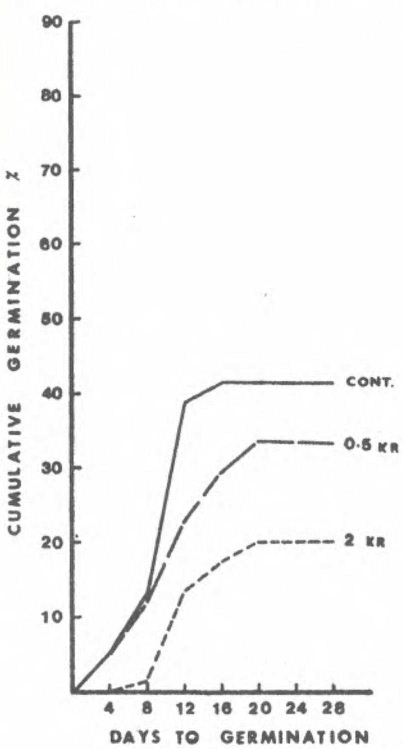
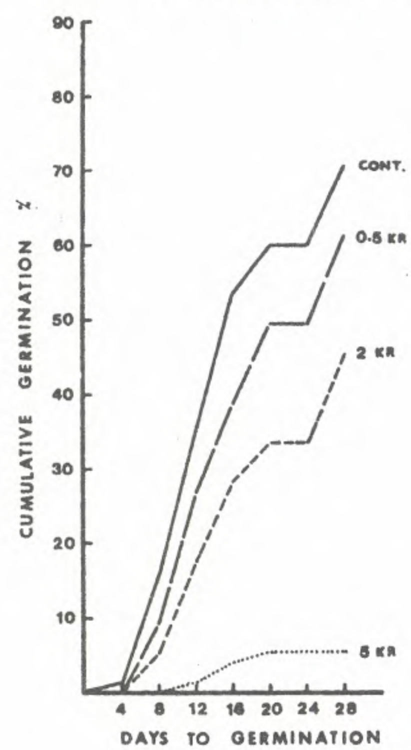


FIG. 12. **Western hemlock**



Pinus strobus has been reported to be a highly sensitive plant. A dosage of 2,000 r. of X-rays gave lethal survival effects to longleaf and slash pine seeds (Snyder et al., 1961). Gustafsson and Simak (1958) observed a reduction in germination by 90% in spruce after exposing dry seeds to 1,200 r. May and Posey (1958) reported p 10% germination of dry *Pinus elliottii* seed after irradiation of 25 kr. from Co60 source, and about 90% germination after seeds received 5 kr. These are a few examples which show that there is a considerable variation among different species of pines and this variation in many instances reflects the methods used.

The LD50 for Douglas-fir seeds from the interior was higher by about 3 kr., than that from the coastal part of the province. This indicates that the radio-sensitivity of Douglas-fir seeds differs according to the source of seeds. Laffers (1960) found that seedlings of *Picea abies* from higher elevation sources were more resistant to y-rays than those of low level origin. Ohba and Simak (1961) concluded that *Pinus sylvestris* seeds from the provenances with a short growing season (northern latitude and higher altitude) were more radiosensitive than seeds from lower altitudes and southern latitudes.

Accelerated germination of X-rayed seed was first reported by Maldiney and Thonvenin in 1898 (cited by Sax, 1963). In this experiment, there was an increase in the germination percent of Douglas-fir seeds when irradiated with doses up to 2,000 r. This is in agreement with the reported slight stimulating effect on germination of *Pinus banksiana* resulting from 5,000 r. y irradiation (Yim, 1963). For soaked and unsoaked Japanese red pine seed, exposure to fast neutrons at certain doses increased germination, survival, and growth (Sato and Nishina, 1951). Tralau (1957) reported stimulation in germination rate and growth from spruce seed y irradiated, mostly at 150-300 r. Increased germination or growth from y irradiated conifer seeds has been reported at 300- 400 r. (Muller-Olsen and Simak, 1954; Muller-Olsen et al., 1956; and Simak and Gustafsson, 1953). Vidakovic (1960) noted increased "germination power" of pine and spruce seed from y irradiation at 748 r. or lower.

Beaty and Beaty (1956) discovered that an X-ray dose that stimulated at 5 r./min. was inhibitory at 15-50 r./min. Neither Gustafsson and Simak (1958) nor Baldwin (1936) could detect stimulation from y irradiation of conifer seed.

A significantly higher germination percent of stratified seeds than the unstratified ones was recognized in Douglas-fir and Sitka spruce. The reverse occurred in western hemlock. Mergen and Cummings (1965), working with *Pinus rigida*, reported moist stratification caused an increase in germination that was paralleled by a decrease in cytological damage at all exposure levels they used. A three-week stratification period after irradiation of dry seeds up to 1,800 r. did not affect germination of *Pinus rigida*, Yim (1964).

Tasher (1919) was the first to demonstrate a post irradiation effect in resting seeds. Stadler (1930) showed that the percentage of induced mutations was not altered by storing for two weeks at temperatures ranging from 5 to 38 C.

The decrease in the germination of western hemlock after stratification could be due to "storage effect." Sax (1941) reported increased chromosomal abnormalities after storing onion seeds for a week after irradiation. Adams et al. (1955) reported increased damage and chromosomal aberrations after storing X-ray treated barley seeds for periods up to six weeks. Gustafsson (1937) indicated that storage of seeds for a year after irradiation increased the X-ray damage. On the other hand, Caldecott indicated in 1955 that he was unable to observe any storage effect in seeds.

SUMMARY

The effects of γ irradiation at different doses on the germination of Douglas-fir (from two different provenances, coastal and interior), Sitka spruce, and western hemlock were investigated. The following points could be drawn from this preliminary report:

1. Doses above 5,000 r. had deleterious effects on seed germination for all species studied.
2. Stratification of seed after irradiation resulted in higher germination percent over the unstratified seeds in the case of Douglas-fir and Sitka spruce, but not in western hemlock.
3. Doses of 500 r. and 2,000 r. significantly stimulated the germination of Douglas-fir seeds from the interior region.
4. The LD_{50} values varied considerably among the species and they could be arranged in the order of increasing radiosensitivity as follows: (1) Douglas-fir (interior provenance); (2) Douglas-fir (coastal provenance); (3) western hemlock; (4) Sitka spruce.

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