Chairman: D. P. Fowler

CHRONIC IRRADIATION STUDIES IN CONIFERS - A PRELIMINARY REPORT[1]

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ABSTRACT

Dry seeds for six species of conifers, including jack pine, red pine, Scotch pine, white pine, black spruce and white spruce, were exposed to a chronic regime of gamma irradiation from 60 cobalt. The dose-rate employed was 50 R per 24-hour day; the total dosages varied from 50 R to 12,600 R. The irradiation time varied from one day to 252 days, depending on the total dose administered.

Samples of 100 irradiated seeds were germinated in the laboratory: 300 irradiated seeds were planted at the Orono Tree Nursery in November and grown there for one year. Suitable controls were employed.

Thirty-day germination tests in the laboratory demonstrated a ten-fold range of sensitivity; jack pine germinating to 9.5 KR and white pine germinating to only 1.2 KR. In the nursery, an increased sensitivity under environmental stress reduced .the range of sensitivity to five-fold; jack pine surviving to 3.4 KR and white pine to 0.75 KR.

Samples of surviving seedlings were taken at the end of one growing season. The seedling weights did not differ significantly from the control populations. The major effect of chronic irradiation was higher lethality than under an acute regime. The surviving seedlings were normal in appearance and weight. It is concluded that, under a chronic regime, seeds do not survive at all with manifest damage.

INTRODUCTION

Woody spermotophytes, especially gymnosperms, are more sensitive to ionizing radiation than herbaceous species (Sparrow and Sparrow 1965).

The lethal dose for pine trees in a gamma-field is given by Sparrow as 1.0 - 1.5KR (1965). This holds for full grown trees and for young seedlings (Sparrow and Sparrow 1965). A variety of lethal doses have been reported for seed ranging from 1.0 KR soft X-rays for Scotch pine (Simak and Gustafsson 1953), through 2.0 KR for longleaf and slash pine seed (Snyder et al. 1961) to over 13 KR for slash pine (May and Posey 1958). Seeds irradiated in the cones on trees in a gamma field were reported to survive 16.0 KR given at a dose-rate of 130 R per day with no effect on germination (Mergen and Johansen 1961).

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Natarajan and Maric (1961) reported a 'time-intensity' factor in irradiated dry barley seed. Seed stored post-irradiation showed more damage to germination and seedling weight than seeds germinated immediately. This effect was more pronounced with low than with high dose-rates.

These studies were conducted to test whether chronic irradiation is as damaging, more damaging, or less damaging than acute irradiation.

MATERIALS

The seed of the six species of conifers was supplied by the Ontario Department of Lands and Forests from their Tree Seed Plant at Angus, Ontario. The seed had been extracted from the cone, dewinged, and pelleted with "Captan", a fungicide. Sets of 100 seeds for germination tests, and 300 seeds for field planting were sorted for uniform large size, and counted into small brown envelopes. The seed were stored in the refrigerator at 6 ± 0.5°C until used.

Table 1.--Seed particulars.

Species	Common name	Moisture content	Year harvested
Pinus banksiana Lamb	jack pine	4.1%	1962
Pinus sylvestris L.	Scotch pine	6.1%	1961
Pinus resinosa Ait.	red pine	3.9%	1962
Pinus strobus L.	white pine	2.4%	1962
Picea mariana Mill.	black spruce	3.7%	1961
Picea glauca Moech.	white spruce	2.8%	1962

Tests at the end of the experiment indicated that all seed had equilibrated to 6.0 ± 0.5% moisture by weight during storage prior to irradiation.

METHODS

Radiation facility and dosimetry.

A one Curie Cobalt-60 source was used to irradiate the seeds. The source is located in an enclosed, undisturbed room in the sub-basement of the Medical Building at the University of Toronto. The castle is located in a 2-foot hole in the floor, and the Cobalt pencil is welded to the lid of the castle, which is raised and lowered by a pulley system, operated from outside the room (fig. 1).

For dosimetry, the 25 R probe with build-up cap, and a Victoreen R condenser chamber (Model 70), was used. The gamma flux was measured at 25 cm. from the center line of the source. The required dose-rate of 0.035 R per minute to give 50 R per day was calculated using the "Inverse Square Law". Dosimetry was repeated once a month, and the distance adjusted as needed to compensate for decay,

Irradiation.

Fifteen total dosage were given during the summer of 1964. Exposure dosages and starting times are shown in table 2.

Table 2.--Exposure dosages and starting times.

Dose (KR)	Exposure time (days)	Starting date (1964)	Dose (KR)	Exposure time (days)	Starting date (1964)
12.6	252	Feb. 27	1.5	30	Oct. 2
11.2	222	Mar. 27	1.2	24	Oct. 8
9.5	190	Apr. 29	0.95	19	Oct. 17
8.1	163	May 26	0.75	15	Oct. 21
6.6	133	June 26	0.50	10	Oct. 23
4.3	86.5	Aug. 11	0.25	5	Oct. 28
3.4	69.5	Aug. 28	0.05	1	Nov. 1

All seed was removed on November 2, 1964, Sets of 100 seeds were retained in the laboratory for germination tests and 300 seeds were planted at the Orono Tree Nursery, Orono, Ontario, within the first week of November, 1964.

Seed germination studies.

Seeds for the various treatment groups were placed in sterile petri plates, moistened with distilled water, and incubated at 23 ± 1 °C for thirty days. Counts were taken every three days up to thirty, at which point any seeds not germinated were considered non-viable.

White pine and white spruce were given 50 days cold-moist stratification (5 ± 1°C) prior to the germination test (Graber, 1965).

Planting site and planting procedure.

The nursery is situated at Orono, Ontario. The soil in the area where the experiments were carried out is a sandy loam, with a neutral pH (6.4 - 7.4) and an adequate level of organic material (1.5-3.0 percent). Soil tests indicated medium levels of all nutrients with the exception of magnesium. No fertilizers were employed in this area during the experimental period.

The seed were broadcast by hand at a planting density of 75 per square foot. The seed was covered with 1/4" of red sand and rye-straw mulch and light screens placed on the beds for the winter. In the spring, the light screens were raised to the standing position and the straw removed as the seedlings started to emerge.

Parameters employed in assessing radiation effects.

1. Thirty-day germination.--Seeds whose radicle had attained the length of the seed coat were considered to have germinated, all others were considered to be non-viable.

2. Emergence.--The number of seedlings emerging was counted in the first week of June, 1965.

3. Survival.-- The number of seedlings surviving one growing season was counted in October, 1965.

4. Seedling weights.--Ten seedlings for each of the experimental groups were removed in October, 1965. The fresh and dry weights were measured individually and the mean weight for each treatment group is as recorded.

RESULTS AND OBSERVATIONS

Germination under laboratory conditions (30-days).

No species germinated over the full. range of dosages employed; at the lower doses, all species appeared normal, and germinated at a rate comparable to the control seed, At higher doses the number of seed germinating decreased with increasing dose (fig, 2). The range of doses over which this decrease was observed differed from species to species, and reflects their order of sensitivity, as listed for the lethal doses to 50 and 100 percent of the population (table 3).

Table 3.--Germination (30 days).

Species	LD/50 (KR)*	LD/100 (KR)
Jack pine	7.0	9.5
Black spruce	5.8	8.1
Red pine	4.0	9.5
Scotch pine	0.95	3.4
White spruce	0.90	1.5
White pine	0.65	1.2

* LD50 from linear plots; LD100 observed.

Emergence in the field.

Field germination is always lower than laboratory germination, the average ratio of the two being about 0.5 (Stoeckeler, J. H., and G. W. Jones, 1957). In this experiment, the ratio of the field tests to laboratory germination was less than 0,5 varying with species. This indicated severe environmental stress on the control seed.

At low doses, the number of irradiated seedlings was equal to or greater than control, This 'stimulatory' effect was more pronounced in the field material than in the laboratory tested material. Since the variation of the control is not known, there is no way of testing the significance of this apparent increase in number over control,

At higher dosages the numbers of seedlings emerging decreased with increasing dose, Dosages required to produce either 50 or 100 percent lethality were lower for all species when compared to germination data under laboratory conditions (fig, 3).

Survival in the field (1-year).

The numbers of seedlings surviving one growing season were fewer than the number emerging, This was true of both the irradiated and non-irradiated material. With the irradiated seed, the higher dosage groups were more severely affected than at the lower dosages, some being eliminated entirely (table 4, fig. 4)

The pronounced stimulatory effect (values exceeding control) seen on the survival graph is dramatic, Its significance could not be tested, however, since the population variance for this experiment was not known,

Jack pine was still the most resistant at the end of one growing season, and white pine the most sensitive. Black spruce exhibited a much reduced emergence and survival when compared to laboratory germination studies. This was ascribed in pa to the fact that it is grown out of its provenance at Orono. Red pine survival wa extremely poor due to damping-off at the nursery (fig. 4).

The range of sensitivity was, by one year, reduced five-fold.

Table 4.--Survival data (1-year).

Species	LD/50 (KR)	LD/100 (KR)
Jack pine	3.0	3.4
Red pine	0.75	0.75
Black spruce	0.20	0.95
White spruce	0.61	0.75
Scotch pine	0.73	0.95
White pine	0.57	0.75

LD/50 calculated from linear plots; LD/100 observed.

Seedling weights (1-year).

The control population tended to be homogeneous for both fresh and dry weights, with most of the values grouped around the mean. The mean and the standard deviation were calculated for all samples, and the 95 percent confidence limits were constructed using the 't' distribution. The means of the irradiated samples fell within the control population in all cases but two. White spruce irradiated with 0.5 KR were significantly smaller ($p < 0.01$) for both fresh and dry weights. Scotch pine plants at 0.05 and 0.25 KR were significantly larger ($p < 0.05$) for both fresh and dry weights.

DISCUSSION

High lethality and reduced tolerance to environmental stress are two of the main effects observed in these experiments. Sparrow (1965) has stated that the effect is the same whether the dose is delivered chronically or acutely; but, that the total dose required to produce the same effect generally increases as the exposure time increases. In our case, however, chronic irradiation proved to be more damaging -- all parameters considered. The results for germination and survival indicate that the conifer seed is very sensitive to ionizing radiation and corresponds to the lower estimates found in the literature for acute dosages (Simak and Gustafsson, 1953; Snyder et al., 1961). Clark et al., 1965, reported that the same six species had a lethal range of 3260 to 8640 R for germination, and 1720 to 4730 R for survival at the end of one growing season. In the present studies 100 percent lethality was produced at lower dosages. Before it is concluded that the difference can be assigned to dose-rate effects, it should be noted that the two experiments were completed one year apart and the seed was older at the time of irradiation and planting. Although germination tests indicated no loss in viability, older seeds are more sensitive to radiation (Nilan, 1956; Sax and Sax, 1964).

The increased lethality could be related to a storage factor. Such a factor has to be considered since the radiation stress was administered over a prolonged period of time (up to 252 days) under dry conditions. Long dry storage post-irradiation and prior to planting is known to potentiate radiation damage (Adams and Nilan, 1958; Curtis et al., 1958; Natarajan and Maric, 1961), Fall planting and winter in the field was equivalent to stratification which would reduce radiation damage (Snyder et al,, 1961).

Moisture content at the time of irradiation is critical (Mergen, 1965; Ohba, 1961; and Snyder et al,, 1961). The seeds varied in moisture content from 2,8 to 6.1 percent on shipment from source, After storage of one year in the refrigerator, all o ces had equilibrated to 6 percent moisture by weight, In the irradiation room, ¯me relative humidity was low and the low moisture content was maintained, Ohba (1961) found, with Pinus densiflora, that a 13 percent moisture content increased the radio-resistance over lower and/or higher moisture contents, Considering then age, moisture content, and storage the seeds were potentially radiosensitive, and radiation proved lethal to many of the treatment groups,

A second radiation effect relates to a reduction in vegetative growth (Sparrow, 1965)o Reduced growth in our studies was not observed. The surviving seedlings, grown from the irradiated seed, were within the distribution of weights for the control seedlings

The stimulatory effect of irradiation at low doses on yield and plant size has been reported and questioned for many years (Johnson, 1936; Sax, 1963), In our experiment, the increase in numbers that occur at the lower dosages involves approximately one-third of the surviving treatment groups and may be the results of increased drought resistance (McCormick and McJunkin, 1965), Parallel data should indicate whether or not the above results are significant,

SUMMARY AND CONCLUSIONS

Seeds of six species of conifers were exposed to chronic gamma irradiation (50 R per day). Damage was assessed on germination, field emergence, one year survival, and seedling weight, Laboratory germination tests indicated a 10-fold range of radiosensitivity which environmental stress reduced to five-fold in one year. The surviving seedlings were of normal size, Since five of the six species failed to survive more than one KR, it is concluded that chronic irradiation is more damaging to dry dormant conifer seeds than is acute irradiation,

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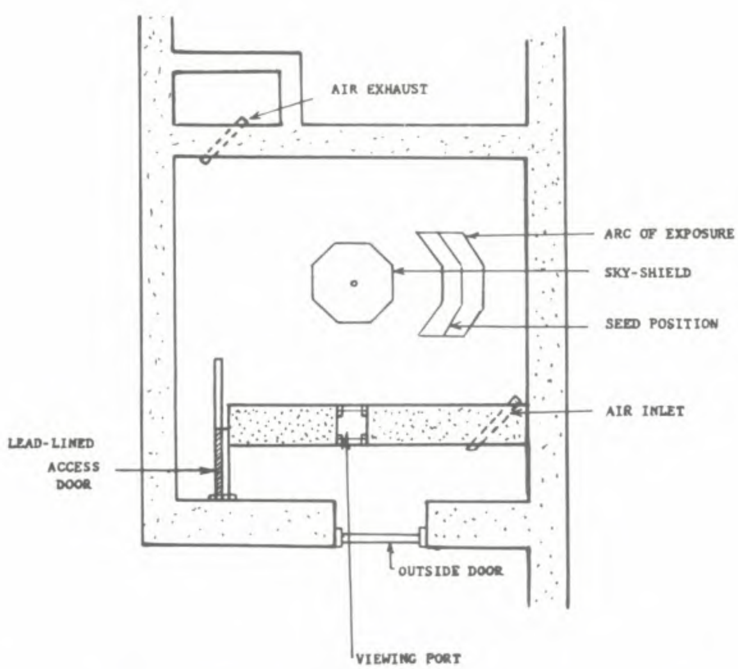

Figure 1.--The floor plan of the radiation facility in the Medical Building, University of Toronto, showing the seed position in relation to the cobalt source.

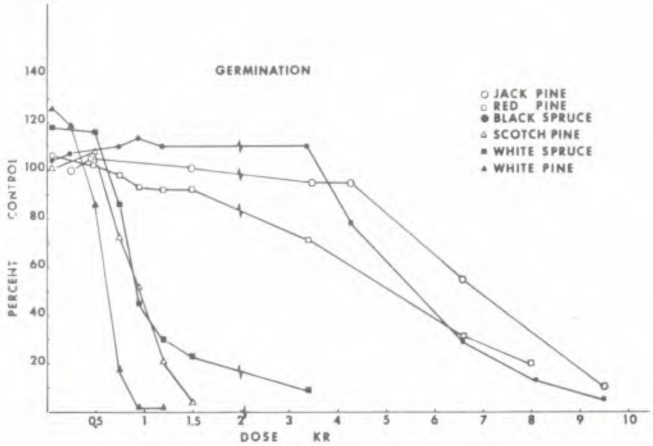

Figure 2.--The dose response curve for laboratory germination. The number germinating is plotted as percent control versus dose (KR).

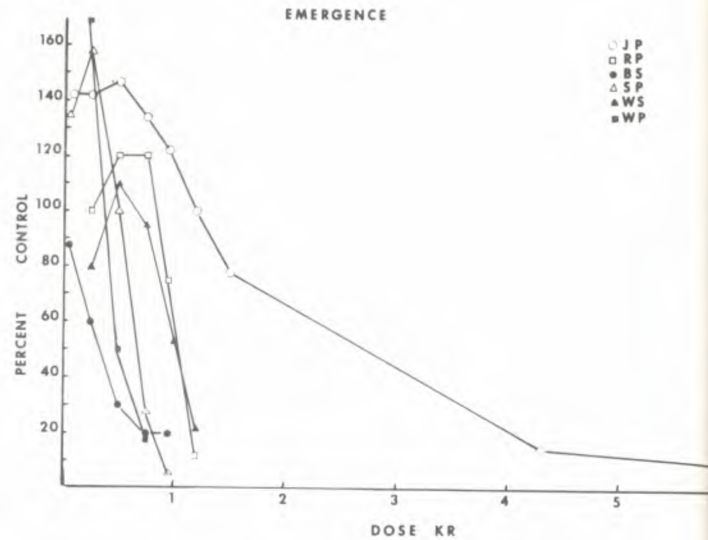

Figure 3.--The dose response curve for field emergence showing the number of seedlings emerging as percent control versus dose (KR).

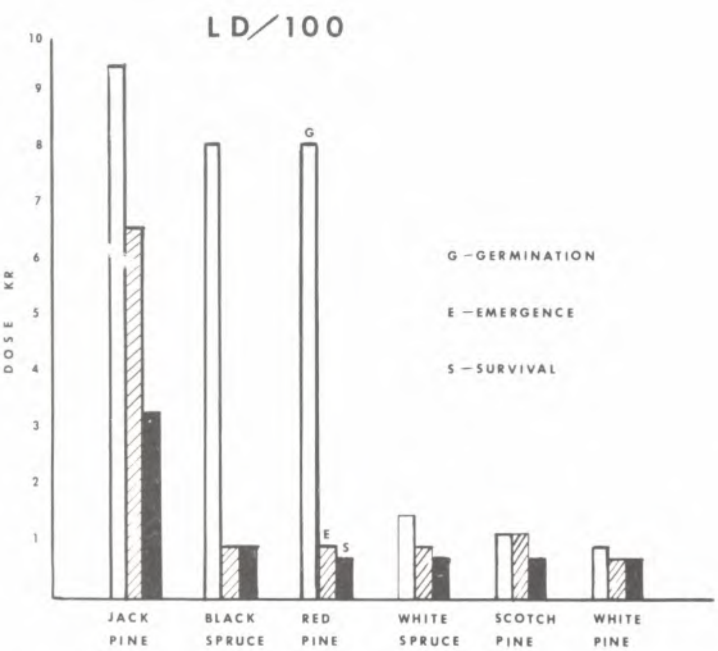

Figure 4.--Histogram of the 100 percent lethal doses for the three parameters; germination, emergence and survival. This illustrates the sharp increase in lethality with the addition of environmental stress.

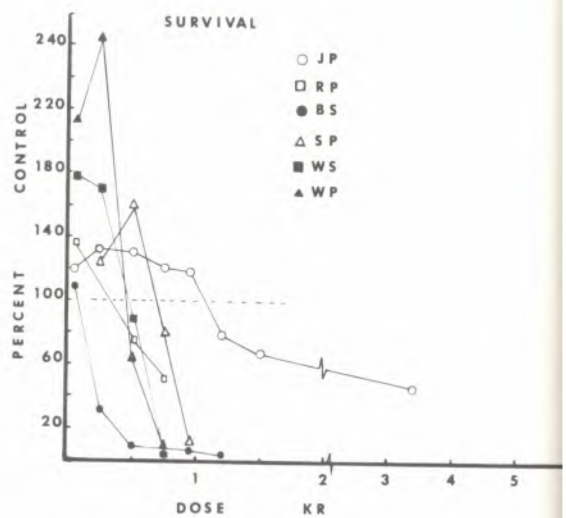

Figure 5.--The dose response curve for survival to the end of one growing season; the number surviving is plotted as percent control versus dose (KR).