to apparently normal green. They repeat this reaction every year.

Cotyledons are always green in the progeny of reciprocal crosses between Wogon-Sugi and normal Sugi. Meanwhile, primary leaves of almost all, i. e. 99 per cent of the seedlings derived from crosses of *Wogon*-Sugi(female) X normal Sugi(male) are normal green. And more than 90 per cent of the seedlings raised from normal Sugi(female) X *Wogon*-Sugi(male) and selfing of Wogon-Sugi had primary leaves and sprouts with white to yellowish white (sometimes, light green) in color. In the same crossings, about 5 per cent (in average) of the seedlings showed chimeric primary leaves and eventually developed into chimeric plants, and there were a few per cent of normal seedlings. The ratios of the seedlings of chimera and normal type after crosses of normal Sugi(female) X *Wogon*-Sugi(male) varied among female parents.

After investigation of segregation on a recessive marker gene, theoretically expected ratios of 3 : 1 (selfing of heterozygous F, hybrids), and 1 : 1 (backcrossing to homozygous female with F, hybrids) are secured and no specific gametic or zygotic elimination is shown for the gene. By these breeding experiments, Wogon type seedlings with homozygous for the recessive gene were obtained.

After a grafting experiment, non transmissibility of the trait of *Wogon*-Sugi was proved. In addition, by electron microscopic observation, underdeveloped lamellar system in the Wogon type chloroplast is shown.

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# Polycross Analysis of Pollen Radiosensitivity in Picea glauca[1])


By D. B. HOUSTON and G. R. STAIRS[2])


## Introduction

The sensitivity of forest trees to ionizing radiation has been studied extensively during the past few years. Whole tree and seed tolerances to chronic and acute irradiation have now been adequately defined for several species, especially in the genus Pinus. To date, however, relatively few studies have been aecomplished with forest trees species in the realm of gametic, or pollen, irradiation. Of those investigations reported, only a small proportion have been oriented toward definition of pollen radiosensitivity for subsequent employment in a breeding program.

Breeding programs must first be preceded by studies to determine the radiosensitivity of the pollen in the desired species, and to include the delimitation of exposures most suitable for the specific purpose. The purpose of the study herein reported was to characterize the radiosensitivity of white spruce (Picea glauca [MOENCH] Voss) pollen through the use of a polycross mating scheme as tested in two plantations in different environments. Radiosensitivity was judged by germination percentages of seed derived from the polycross, electron spin resonance spectroscopy of irradiated pollen, and germination of irradiated pollen in vitro. The study was designed to evaluate nuclear radiosensitivity, cytoplasmic radiosensitivity, and the possibility of parthenogenesis induction through the use of irradiated pollen.

## Review of Literature

Studies of chronic and acute ionizing radiation effects on forest trees have been conducted with seeds, pollen, and somatic tissues. Recent literature surveys of these studies have been published by ERIKSON et al. (1966) and by LYNN (1967). In addition, acute gamma irradiation survival data for 28 species of woody plants, including many forest tree species, have been compiled by SPARROW et *al.* (1968).

Pollen irradiation has received somewhat less attention than other aspects of forest tree research. This situation may be due to difficulties in handling arising from the limited span of viability and hygroscopic nature of the microspore. BREWBAKER and EMORY (1962) reviewed irradiation studies with mature angiosperm pollen, finding $LD_{50}$ values for pollen germination in vitro ranging to 550KR (kiloroentgens), and a median lethal dose for germination of 250KR. In contrast, a median dose of only 250R (roentgens) was reported for pollen tube divisions. VIDAKOVIĆ (1963) fertilized Pinus sylvestris with P. nigra pollen irradiated at 800R to 1200R and obtained putative hybrid progeny from this otherwise incompatible cross. The investigator theorized that gamma radiation stimulated pollen tube growth and fertilizaticn subsequently occurred, or, as a result of the radiation, certain chemical changes occurred in the pollen thereby stimulating development of the female gametophyte and fertilization. Naturally occur-


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ring hybrids as well as artificially produced hybrids of these two species have been reported previously in the literature (VIDAKOVIĆ, 1958; RIGHTER and DUFFIELD, 1951; WRIGHT and GABRIEL, 1958). It is possible that the author had experienced difficulty in otherwise obtaining progeny from the mating of the species under controlled hybridization conditions. High level acute irradiation of pollen from *Pinus rigida* and *P. strobus* produced no significant effect on germinative ability *in vitro*, even after exposure to 300KR (MERGEN and JOHANSEN, 1963). No significant decrease in pollen tube lengths were found, but at the highest level of exposure the results were more variable than at lower levels. Tube length *in vitro* was not correlated with the chronic exposure of *Pinus rigida* trees utilized in another portion of the study, indicating a higher cytoplasmic than nuclear tolerance to gamma irradiation. Similar findings have been reported by FUJIMOTO *et al.* (1964). Mature pollen of *Pinus densiflora* and *P. thunbergii* was irradiated to 75KR with no significant effect on *in vitro* germinability. However, pollen tube length decreased with increasing dosage.

RUDOLPH (1964) investigated the effect of gamma irradiation (0—800R) of pollen on seed characteristics in *Picea glauca*. A stimulatory effect on seed set and seed viability was noted, with the effects being most pronounced at 600R. It was suggested that the $LD_{50}$ dosage for white spruce pollen is higher than 800R but possibly lower than the $LD_{50}$ for pollen of many other plant species. No effect was noted when mature pollen of three *Quercus* species was acutely irradiated at 100 KR (STAIRS, 1964). At 300KR both germination and tube lengths were depressed. The acute irradiation of microsporangiate strobili at different stages of meiosis was also reported, and a range of 1KR to 4KR suggested as an appropriate exposure for male buds to be used in a mutation breeding program. ERIKSON *et al.* (1966) studied the induction of a mutant "waxy" phenotype in *Larix leptolepis* pollen by irradiation as a means of measuring mutation rates and estimating the dispersal of mutations in forest tree populations. Waxy pollen grains were induced after 24 hours gamma irradiation ($Cs^{137}$) of the pollen mother cells. The data indicated the mutation rate reached a plateau after as low a dose as 90 rad.

The effect of radiation on the properties of *Pinus nigra* and *P. sylvestris* pollen has been studied by DJURBABIĆ *et al.* (1967). Analysis revealed the concentration of reducing sugar in *P. sylvestris* pollen to be more than double that of *P. nigra*. After irradiation at 1000R, the concentration in *P. sylvestris* dropped to nearly equal that of unirradiated *P. nigra*. It was proposed that the amount of reducing sugars in the pollens could be a significant factor contributing to the incompatibility of the species, especially in light of VIDAKOVIĆ's previously successful radiation-induced hybridization of these pines. STAIRS and TROENDLE (1960) reported an $LD_{50}$ of about 200KR, with less than one percent germination at 600KR and none at 800KR, for acutely irradiated pollen of four conifer species.

Electron spin resonance (ESR) spectroscopy has been extensively utilized in the field of radiation biology over the past decade. The general physical theory and mechanics of ESR have been dealt with in several presentations (see WERTZ, 1955; COOK and WHIFFEN, 1962; ANDROES and CALVIN, 1962; SQUIRES, 1964) and hence will not be discussed here. For a recent comprehensive survey of ESR studies accomplished in the field of radiobiology, the reader is referred to ZIMMER and MÜLLER (1965). As these authors point out, few generalizations or regularities have emerged from the research that has been reported.

It is now generally accepted that the formation of organic free radicals (i. e. centers of ESR absorption) can be induced in biological material by subjecting it to ionizing radiation. Biological damage attributable to ionizing radiation is thought to be the result, at least in part, of the reaction of these free radicals with each other or with neighboring biologically important molecules.

The majority of the investigations reported since the technique was introduced have been primarily concerned with the elucidation and characterization of the processes by which ionizing radiation leads to biological damage. Much of the ESR work accomplished to date has involved the irradiation of "complete" biological entities, such as seeds, spores, and bacteriophage (see EHRENBERG and EHRENBERG, 1958; CONGER and RANDOLPH, 1959; CONGER, 1961; LÖFROTH *et al.*, 1964), or biologically important substances, such as DNA, proteins, and amino acids (see DORLET *et al.*, 1962; SALOVEY *et al.*, 1963; PRYDZ *et al.*, 1961; GORDY and SHIELDS, 1960; MÜLLER and KÖHNLEIN, 1964 a, 1964 b; MÜLLER, 1964).

Positive identification of the free radicals produced by ionizing radiation has been a difficult and unrewarding task in most instances due to the chemical complexity of the materials studied. However, a few general mechanisms have been suggested to explain the manner in which biological damage may occur (see PATTEN and GORDY, 1960; GORDY and SHIELDS, 1960; GORDY *et al.*, 1965). ESR evidence from these studies indicates that there are two principal sites of radiation damage to proteins: (1) the alpha carbon in the polypeptide backbone; and, (2) the sulfur atom of a side chain of a cysteine or cystine residue. Usually sulfur forms a disulfide bridge, either between different parts of a polypeptide chain or between adjacent chains, while the alpha carbon is located at the bend point in the coiled protein molecule. Thus any disruption of the molecular configuration at these two sites, as by ionizing radiation, leads to a conformational change in the protein structure and a subsequent alteration of its function. ESR analysis of gamma-irradiated DNA and its constituents suggests that hydrogen-addition reactions on the ringed groups, especially on the thymidine groups, are possibly of great significance in radiation damage to this nucleic acid.

To the author's knowledge, the ESR technique has not been previously utilized in forest tree irradiation studies. Examples of successful applications of ESR to the study of radiation-induced biological damage in plant tissues are found in reports by EHRENBERG and EHRENBERG, 1958; TANOOKA, 1966; BHASKARAN, 1964; and BHASKARAN and KÖHNLEIN, 1964. In addition, several studies (e. g. CONGER and RANDOLPH, 1959; CONGER, 1961) have utilized ESR spectroscopy to correlate free radical production by ionizing radiation and biological damage occurring post-irradiation.

## Materials and Methods

Two plantations of white spruce *(Picea glauca)* were selected for study. The original seed source of the planting stock could not be ascertained as the seed was collected from existing plantations in several New York State forest districts and then mixed for sowing in the nursery. The plantations were about 15 years old; Plantation No. 1, at McGraw, New York, is located approximately 100 miles south of Plantation No. 2, at Turin, New York.

*Controlled Pollinations, Seed Collection and Germination*

A polycross mating scheme was utilized in the study to provide seed for subsequent germination trials. Thus the selected females in each plantation were pollinated with a mixture of pollens collected from males in the same or both plantations. The polycross method was employed to provide a broad genetic base for the study, thereby minimizing the effect of genetic variability between pollen parents that might obscure treatment differences.

Thirty trees in each plantation were chosen on the basis of flower abundance to serve as the female parents. Each tree was numbered and non-woven-cloth pollinations bags were applied; approximately eight female strobili were isolated per bag. Microsporangiate strobili were collected from 15 trees in each plantation. The pollen was extracted in the laboratory, mixed, and stored in glass vials over a

silica gel dessicant at 0°—1° C. Moisture content of the pollen was maintained at 2—3 percent during storage.

Only six pollination bags could be placed on a tree; therefore the pollen radiation exposure levels were divided into three treatment groups as follows:

Group A: 4KR, 8KR, 16KR, 32KR, Control
Group B: 6KR, 10KR, 12KR, 14KR, 18KR, Control
Group C: 50KR, 100 KR, 150KR, 200KR, 400KR, Control

A given treatment group was applied to each of 10 female trees in both plantations. Thus, a representative female parent was pollinated with all the exposure levels plus the Control (unirradiated) in a given treatment group, so that a total of 10 replications of every exposure level appeared in each plantation. Ten bags per plantation were left unpollinated to test the isolation techniques. Trees in each treatment group were distributed randomly among all selected trees in the plantations to minimize the effects of site differences.

At the time of female receptivity in the field, the pollen was irradiated at a rate of 300R per minute from a $Co^{60}$ gamma radiation source of approximately 600 curies. During irradiation the pollen remained in the glass vials, with an average temperature range of 20° to 22° C. Dosimetry was determined using a Victoreen Roentgen Rate Meter with an ionization chamber preamplifier probe assembly rated at ±5 percent accuracy. With the exception of the 4KR level, the pollen vials were turned 180° once during the irradiation period to insure more complete exposure.

The females in Plantation No. 1 were pollinated with pollen from males in the same plantation. Because pollen from Plantation No. 2 was limited, those females were pollinated with a mixture of pollens remaining from the No. 1 area, plus the 15 No. 2 area males, and three males from a similar plantation at East Wooster, New York. Pollens from other than Plantation No. 2 comprised less than 10 percent of the total. All bags were repollinated three days after the initial pollinations, and the bags removed 14 to 18 days later.

The mature cones were harvested in the last two weeks of August, dried, and the seed extracted. The extracted seed was dewinged and cleaned of foreign material; however, the empty seed was not removed at this stage. The seed was stored at 4° C prior to the germination trials.

A preliminary test of non-stratified versus stratified white spruce seed was conducted in a JACOBSEN germinator to test the response of the two treatments. After 24 days, germination averaged 77.5 percent for the non-stratified seed as opposed to 70.7 percent for the stratified seed. Therefore all succeeding germination trials were conducted without stratification. These results are in accord with the recommendations for white spruce seed previously made by HEIT (1963). Subsequent germination trials were conducted in the JACOBSEN germinator using a 12 cm. filter paper media and glass covers to maintain humidity relations. Prior to each trial the germinator was sterilized with 95 percent alcohol, and the filter paper soaked in methylmercury diciandiamide (1 : 4,500) to prevent fungus growth during early germination. Artificial light (14-hour day) and alternating 30°—20° C (day-night) temperatures were maintained during the germination period.

Germination trials were scheduled as follows:
1st trial: Treatment Groups A and B — Plantation No. 1
2nd trial: Treatment Groups A and B — Plantation No. 2
3rd trial: Treatment Group C — Plantations No. 1 and 2

Each trial was arranged in the germinator in a randomized incomplete block design with two blocks and 20 circular plots per block. The plots were partitioned into six equal segments, with 25 seeds per segment. This arrangement permitted all treatments plus the Control in a treatment group for a given female tree to appear in a single plot. An individual female tree and its associated treatment group appeared once in each of the two blocks in a trial. Disregarding parentage and plantation, each treatment was thus replicated 40 times over all trials and was represented by a total of 1000 seeds. Germination counts were recorded daily for 30 days. All ungerminated seed was examined at the termination of each trial to determine the percentage of full seed which did not germinate. After germination the seedlings were transplanted into a medium consisting of topsoil, peatmoss, and sand (1 : 1 : 2).

All extra seed in Treatment Group C (50KR—400KR) was sown in the germinator to further test the hypothesis that genetically sterile but physiologically active pollen may induce parthenogenetic development. Approximately 3,800 seeds for each exposure level were subjected to germination conditions, as previously described for 30 days.

Seed germination data were evaluated through the use of regression analysis to test the differences between exposure levels and plantations.

*Pollen Germination Trials*

Pollen germination trials were undertaken to characterize the cytoplasmic radiosensitivity of white spruce pollen over the range of exposures studied, as judged by germination percentages and pollen tube lengths.

The pollen used in the trials was collected in May, 1967, from the original 15 pollen parents in each plantation. It was extracted, stored, and irradiated as previously described, with the exception that 600KR and 800KR levels were added.

Pollen germination was conducted by sowing the pollen on glass slides coated with a 0.7 percent agar medium containing 5 percent sucrose. Cultures were incubated in petri dishes over distilled water at an average temperature of 28° C for 48 hours. A separate germination trial was conducted with pollen from each plantation. Each exposure level was replicated three times per plantation in a randomized complete-block design. Pollen grains were considered germinated if the pollen tube length exceeded the small diameter of the grain. Germination counts were made at the end of the germination period on 100 grains per slide and were expressed as a percentage of normally appearing grains. Pollen tube lengths were measured with an ocular micrometer on 10 tubes per slide at a magnification of 40×.

*Electron Spin Resonance Spectroscopy*

ESR spectroscopy of irradiated and Control white spruce pollen was used to explore the possibility of employing this technique as an indicator of pollen nuclear and cytoplasmic sensitivity.

Pollen was irradiated under the conditions previously described and then transferred to 4 mm O.D. quartz spectrometer tubes. Each quartz tube was flame sealed on one end, filled with approximately 0.05 grams of pollen, and stoppered with cotton.

ESR spectrometry was performed using a Varian X-band EPR Spectrometer, Model V-4502-15, under high power operation with a 100kc field modulation frequency. Spectra were obtained at a sweep range of 100 gauss for each exposure level as well as for an empty spectrometer

tube and represented the first derivatives of the actual absorption curves. Recordings were made at room temperature (approximately 20° C) immediately following radiation exposure.

No attempt was made to assess radical production quantitatively with respect to absolute radical concentrations or to determine the identity of observed radical species. Quantitative determinations of absolute or relative yields of free radicals produced by ionizing radiations are subject to numerous sources of error, and derived values often depend on such parameters as temperature, moisture content, surrounding gas, and radical decay during the period between irradiation and measurement by the spectrometer (ZIMMER and MÜLLER, 1965). Yield determinations are commonly subject to as much as ±50 percent error on an absolute basis, while relative yields can generally be considered at best to represent an accuracy of only ±10 percent (HENRIKSEN, 1963; PIHL and SANNER, 1966). Therefore, quantitative determinations of free radical yields of this nature were not attempted in the present study.

Derived ESR spectra were characterized qualitatively by amplitude measurements and curve shapes at the various exposure levels, as well as by curvilinear regression analysis.

## Results

*Seed Germination Trials*

The germination trials conducted with seed from the irradiated pollen polycross demonstrated a decrease as the pollen exposure level increased. These data are summarized in *Table 1*. An $LD_{50}$ level was noted at approximately 4KR for Plantation No. 1, and 6KR for Plantation No. 2; lethality was obtained at 16KR for Plantation No. 1, while germination averaged 1.11 percent at 18KR in Plantation No. 2. Over all treatments, germination averaged about 10 percent higher for Plantation No. 2 than for Plantation No. 1; however, percent germination for Controls was essentially the same for each area.

The data variances for each plantation were found to be non-homogeneous and a logarithmic transformation was utilized to stabilize the variance. Subsequent regression analyses of the transformed data for each plantation yielded the curves depicted in *Figure 1*. An extrapolation of the regression lines to the zero percent germination point indicated that the upper limit of pollen nuclear viability, as judged by the ability of the pollen to effect fertilization and the subsequent production of viable seed, occurred at approximately 19KR for Plantation No. 1, and 30KR for

*Table 1.* — Seed germination percentages.

| | Plantation No. 1 | | | Plantation No. 2 | | |
|---|---|---|---|---|---|---|
| Treatment | Total % Germination | Germ. as % Sound Seed | % Sound Seed | Total % Germination | Germ. as % Sound Seed | % Sound Seed |
| Control | 55.87 | 85.15 | 65.61 | 53.00 | 91.70 | 57.80 |
| 4kr | 20.33 | 80.49 | 25.26 | 46.20 | 88.17 | 52.40 |
| 6kr | 10.57 | 90.24 | 11.71 | 27.80 | 95.20 | 29.20 |
| 8kr | 5.80 | 80.56 | 7.20 | 15.80 | 89.77 | 17.60 |
| 10kr | 1.43 | 83.33 | 1.71 | 11.60 | 96.67 | 12.00 |
| 12kr | 0.84 | 100.00 | 0.84 | 7.20 | 94.74 | 7.60 |
| 14kr | 0.67 | 100.00 | 0.67 | 2.80 | 100.00 | 2.80 |
| 16kr | 0.20 | 100.00 | 0.20 | 2.44 | 84.62 | 2.89 |
| 18kr | 0.00 | 0.00 | 0.00 | 1.11 | 100.00 | 1.11 |
| 32kr | 0.20 | 100.00 | 0.20 | 0.00 | 0.00 | 0.00 |
| 50kr - 400kr | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

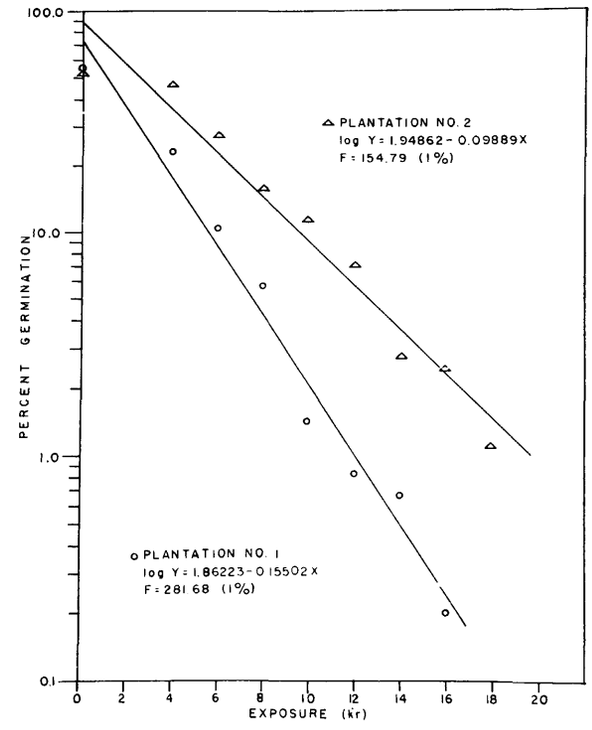


*Figure 1.* — Results of seed germination trials.

Plantation No. 2. Although germination was not tested at discrete pollen irradiation levels between 18KR and 32KR, a general inspection of seed germination trends in the non-transformed data suggested that an average of these two (24—26KR) may be a reasonable representation of lethality for the species.

The regression for each plantation was found to be highly significant (F.01 level) and a TUKEY's Test of treatment means for both plantations *(Table 2)* showed highly significant differences appearing between most treatments. In addition, an F-test of the homogeneity of the two regressions was highly significant, indicating that the two regressions were not estimates of the same population.

One seed (of 1000 tested) germinated at the 32KR treatment level. The authenticity of this individual plant as a true representative of this exposure level cannot be verified as yet. Until its genuineness can be confirmed, it will be considered as a contaminant and will not be considered further in this discussion. The remainder of the 32KR treatment *(ca* 5,000 seeds) sown in nursery seedbeds did not germinate.

None of the approximately 20,000 seeds in the 50KR to 400KR treatment groups germinated, and thus no evidence was obtained to indicate parthenogenetic development.

*Pollen Germination Trials*

Results of the pollen germination trials are presented in *Table 3,* and again showed a difference between plantations. An $LD_{50}$ for percent germination occurred at approximately 400KR for Plantation No. 1 and 300KR for Plantation No. 2. However, germination over all treatments averaged 20 percent higher for Plantation No. 2 than for Plantation No. 1. Germination percentages were the same at 400KR, but at 600 KR and 800KR, Plantation No. 2 percentages were about twice those for Plantation No. 1 (see *Figure 2).*

Growth in length of pollen tubes followed a pattern similar to that for pollen germination, with an $LD_{50}$

*Table 2.* — TUKEY'S test of seed germination means [1]

PLANTATION NO. 1

Treatment Means

| | 16kr | 14kr | 12kr | 10kr | 8kr | 6kr | 4kr | 0kr |
|---|---|---|---|---|---|---|---|---|
| $\bar{X}$ = | 0.20 | 0.67 | 0.84 | 1.43 | 5.80 | 10.57 | 20.33 | 55.87 |
| log $\bar{X}$ = | -0.69897 | -0.17393 | -0.07572 | 0.15229 | 0.76343 | 1.02407 | 1.30814 | 1.74718 [2] |

PLANTATION NO. 2

Treatment Means

| | 18kr | 16kr | 14kr | 12kr | 10kr | 8kr | 6kr | 4kr | 0kr |
|---|---|---|---|---|---|---|---|---|---|
| $\bar{X}$ = | 1.11 | 2.44 | 2.80 | 7.20 | 11.60 | 15.80 | 27.80 | 46.20 | 53.00 |
| log $\bar{X}$ = | 0.04532 | 0.38739 | 0.44716 | 0.85733 | 1.06070 | 1.19866 | 1.44404 | 1.66464 | 1.72428 |

1/ The test was applied to the common logarithms of the mean percent germination values.
2/ Any two means underscored by the same line are not significantly different at the 1% or 5% levels.

observed at approximately 600KR and 400KR for Plantations No. 1 and No. 2, respectively *(Figure 3)*. Pollen tube lengths averaged 7 percent (17$\mu$) longer for Plantation No. 2. Above 50KR, however, the situation was reversed, with Plantation No. 1 tube lengths exhibiting an average differential of 14 percent (21$\mu$) over those for Plantation No. 2 Pollen germination and tube growth, although greatly reduced, were still evident at the highest level of irradiation (800 KR) utilized.

A BARTLETT'S Test of the homogeneity of variance for treatment groups within plantations was not significant (F.05) for pollen germination, and subsequent analyses of variance indicated highly significant (F.01) differences between treatments. Pairwise comparisons of treatment means for each plantation by TUKEY'S method gave the results tabulated in *Table 4*. For Plantation No. 1, no significant differences were evident for treatments through 200 KR.

*Table 3.* — Pollen germination percentages and pollen tube lengths.

| | Plantation No. 1 | | Plantation No. 2 | |
|---|---|---|---|---|
| Treatment | Mean % Germination | Mean Pollen Tube Length (u) | Mean % Germination | Mean Pollen Tube Length (u) |
| Control | 46.00 | 236.9 | 64.67 | 267.0 |
| 4kr | 44.33 | 245.0 | 67.67 | 249.9 |
| 6kr | 41.33 | 231.0 | 63.33 | 232.8 |
| 8kr | 42.67 | 234.2 | 64.33 | 252.4 |
| 10kr | 43.33 | 235.2 | 69.33 | 254.8 |
| 12kr | 41.33 | 227.1 | 66.00 | 259.7 |
| 14kr | 42.00 | 223.7 | 65.33 | 240.1 |
| 16kr | 44.67 | 227.8 | 67.33 | 257.2 |
| 18kr | 45.33 | 244.0 | 70.67 | 254.8 |
| 32kr | 43.67 | 233.5 | 68.33 | 247.4 |
| 50kr | 43.67 | 229.6 | 67.33 | 241.8 |
| 100kr | 45.67 | 234.5 | 64.00 | 221.2 |
| 150kr | 43.00 | 197.7 | 57.67 | 207.5 |
| 200kr | 40.67 | 194.3 | 52.00 | 162.4 |
| 400kr | 21.33 | 148.7 | 21.33 | 120.0 |
| 600kr | 9.33 | 101.2 | 13.33 | 76.0 |
| 800kr | 1.00 | 67.9 | 2.00 | 62.0 |

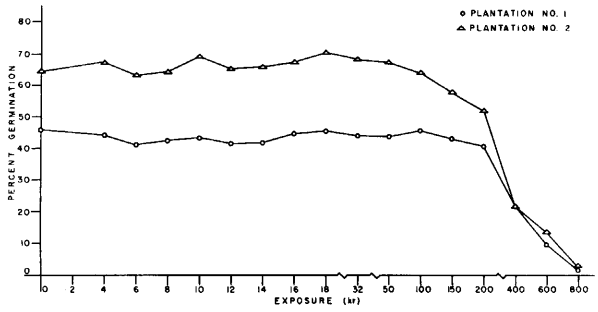


*Figure 2.* — Results of pollen germination trials — percent germination.

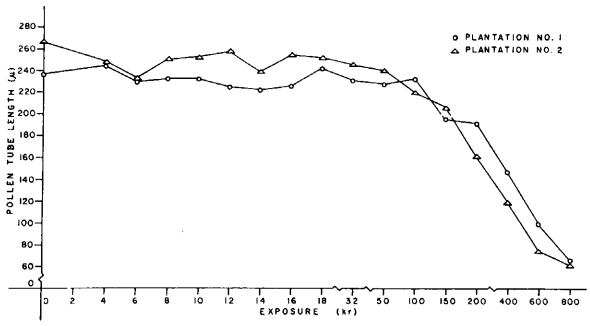


*Figure 3.* — Results of pollen germination trials — pollen tube length.

Highly significant differences did exist between 200KR and 400KR and between 400KR and 800KR. In Plantation No. 2 no differences could be detected for treatments through 100KR, but highly significant differences did exist between 150KR and 400KR, 200KR and 400KR, and 600KR and 800KR.

The variances of the two pollen germination trials were homogeneous, and a subsequent t-test of differences in treatment means between plantations was highly significant.

Homoschedasticity existed between treatment groups within plantations for pollen tube length, and analyses of variance demonstrated the existence of highly significant (F.01) differences between treatments for each plantation. TUKEY'S method for pairwise comparisons of treatment means within each plantation yielded the results enumerated in *Table 5*. While trends were the same in each instance, there were differences between plantations with respect to comparisons between individual treatments. The variance of the two plantations were homogeneous for pollen tube length, and differences in treatment means between plantations, as demonstrated by a t-test, were highly significant.

*Electron Spin Resonance Spectroscopy*

Recorded spectra were obtained at each level of irradiation utilized for the controlled pollination portion of the study and characterized by amplitude measurement and subsequent regression analysis of the derived data. Spectra obtained for an empty spectrometer tube were characterized by a persistent, stable radical signal, presumably resulting from impurities in the quartz, which remained essentially constant in terms of amplitude as measured from the baseline. The same peak also appeared as a distinct signal in each spectrum for the irradiated samples and again remained constant; it is designated in *Figure 4* as the "Control" peak.

*Table 4.* — TUKEY'S test of pollen germination means [1])

PLANTATION NO. 1

Treatment Means

| 800kr | 600kr | 400kr | 200kr | 12kr | 6kr | 14kr | 8kr | 150kr | 10kr | 32kr | 50kr | 4kr | 16kr | 18kr | 100kr | 0kr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\bar{X}$ = 1.00 | 9.33 | 21.33 | 40.67 | 41.33 | 41.33 | 42.00 | 42.67 | 43.00 | 43.33 | 43.67 | 43.67 | 44.33 | 44.67 | 45.33 | 45.67 | 46.00 |

PLANTATION NO. 2

Treatment Means

| 800kr | 600kr | 400kr | 200kr | 150kr | 6kr | 100kr | 8kr | 0kr | 14kr | 12kr | 50kr | 16kr | 4kr | 8kr | 10kr | 18kr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\bar{X}$ = 2.00 | 13.33 | 21.33 | 52.00 | 57.67 | 63.33 | 64.00 | 64.33 | 64.67 | 65.33 | 66.00 | 67.33 | 67.33 | 67.67 | 68.33 | 69.33 | 70.67 |

1/ Any two means underscored by the same solid line are not significantly different at the 1% level; those underscored by the same dotted line are not significantly different at the 5% level.

*Table 5.* — TUKEY'S test of pollen tube length means [1])

PLANTATION NO. 1

Treatment Means

| 800kr | 600kr | 400kr | 200kr | 150kr | 14kr | 12kr | 16kr | 50kr | 6kr | 32kr | 8kr | 100kr | 10kr | 0kr | 18kr | 4kr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\bar{X}$ = 67.9 | 101.2 | 148.7 | 194.3 | 197.7 | 223.7 | 227.1 | 227.8 | 229.6 | 231.0 | 233.5 | 234.2 | 234.5 | 235.2 | 236.9 | 244.0 | 245.0 |

PLANTATION NO. 2

Treatment Means

| 800kr | 600kr | 400kr | 200kr | 150kr | 100kr | 6kr | 14kr | 50kr | 32kr | 4kr | 8kr | 18kr | 10kr | 16kr | 12kr | 0kr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\bar{X}$ = 62.0 | 76.0 | 120.0 | 162.4 | 207.5 | 221.2 | 232.8 | 240.1 | 241.8 | 247.4 | 249.9 | 252.4 | 254.8 | 254.8 | 257.2 | 259.7 | 267.0 |

1/ Any two means underscored by the same solid line are not significantly different at the 1% level; those underscored by the same dotted line are not significantly different at the 5% level.

A weak secondary radical signal was noted in the spectra for the unirradiated pollen sample and may represent free radical production as a function of continuing low level metabolic activity in the dry pollen. As the exposure level of the samples was increased, this secondary peak (designated in *Figure 4* as the "Induced" peak) became more prominent and increased in amplitude with increasing radiation. This signal presumably represented free radical production caused by ionizing radiations and probably was a composite of many different radical species. The Control signal was used as a standard to allow comparison of the Induced peak amplitudes. The induced peak, when plotted as a percentage of the Control signal, exceeded unity in the range 18KR—32KR, and continued to increase through 400 KR, at which point the curve appeared to plateau. A second-degree polynomial regression showed significant (F.01) variation between treatments *(Figure 5).*

## Discussion and Conclusions

An $LD_{50}$ level for the germination of seed derived from the irradiated pollen polycross occurred at approximately 4KR to 6KR. These results differ somewhat from data published previously by STAIRS and TROENDLE (1969) who found no decrease in seed germination at 4KR based on total seed collected. The authors, however, also calculated the regression of percent sound seed on exposure level of pollen and found a significant treatment effect at 4KR.

Numerous studies have demonstrated that moisture content has an important effect on radiosensitivity. CALDECOTT (1955), Ehrenberg (1955), CONGER and RANDOLPH (1959) have shown that the radiosensitivity of barley seeds, as measured by inhibition of seedling growth and frequency of chromosomal aberrations, decreases as seed water content is increased to between 12 and 20 percent. Similar results have been reported by OHBA (1961) and MCMAHON and GERHOLD (1965) for irradiated seeds of *Pinus densiflora, P. sylvestris,* and *P. strobus.* It was suggested that in wet seeds, free radicals produced by ionizing radiation react with water or recombine, and thus do not contribute to the primary effects of radiation exposure. In dry seeds, the mobility of the radicals is impaired due to the absence of a fully aqueous environment, recombination of radicals is lessened, and the probability of reaction with biologically important molecules such as DNA is greater. There is no reason to believe, *a priori,* that the same mechanism does not hold for irradiated pollen. The fact that the pollen utilized in the study reported herein was stored at a relatively low moisture content (2—3 percent) may have been responsible for the slightly higher radio-sensitivity with

respect to seed germination $LD_{50}$'s noted in this study, as compared to that reported by STAIRS and TROENDLE (1969).

The combined seed germination data indicate that the upper limit of germination occurred at pollen exposures of approximately 24KR to 26KR. This suggests that pollen irradiated to or beyond those exposure levels at which seed germination ceases is genetically inert and therefore incapable of fertilization. Based on parameters such as the $LD_{50}$ and the numbers of viable seed produced at various levels of irradiation, an acute exposure level of 6KR to 8KR is recommended for use in a pollen irradiation breeding program for the genus *Picea* and possibly for other genera of the *Coniferales* as well.

The F-test of homogeneity of regression between seed germination regressions (i. e. between plantations) was highly significant (F.01), implying that the two curves could not be considered as estimates of a common regression. In terms of the variables dealt with here, one could say that the relationship between pollen exposure level and seed germination was slightly different for the two plantations. This in turn suggests the possibility of an inherent differential radiosensitivity between the two areas, with Plantation No. 1 exhibiting an apparently greater radiosensitivity than Plantation No. 2. Such an hypothesis can only be considered as speculative at this point, as the exact seed source of each plantation cannot be verified from existing records. It is known, however, that the seed used to establish each area was collected from plantations in two different parts of New York State. The fact that the seed germination percentages for the unirradiated pollen controls of each plantation were almost coincident, as well as the results from the pollen germination trials as discussed below, argue strongly for the existence of a dissimilar radiosensitivity between plantations. OHBA and SIMAK (1961) have demonstrated the existence of intraspecific differences

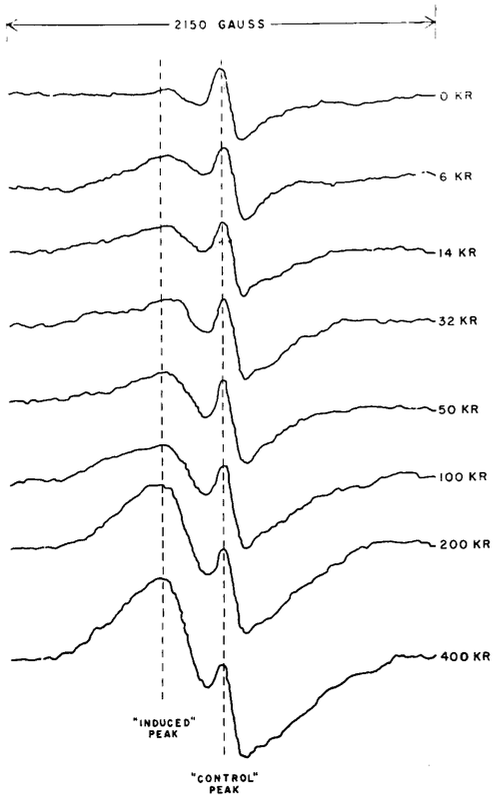


*Figure 4.* — Representative ESR spectra of gamma irradiated white spruce pollen.

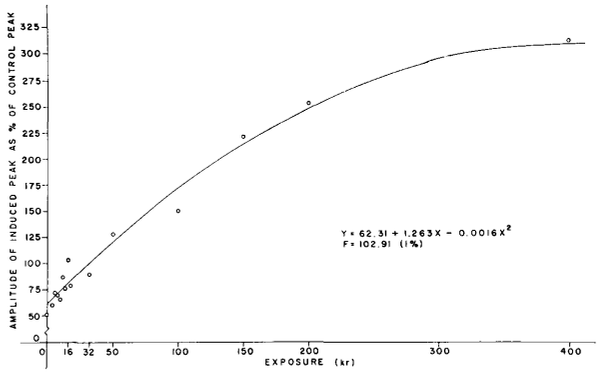


*Figure 5.* — ESR spectroscopy of gamma irradiated white spruce pollen. — Regression of spectra amplitudes on radiation exposure levels.

in radiosensitivity for *Pinus sylvestris,* and such differences may very well be operative in this instance.

It was noted that no seed germination occurred for approximately 20,000 seeds obtained from pollen exposure levels of 50KR to 400KR. While this cannot be construed as infallible proof, it does constitute a test of the hypothesis that genetically sterile conifer pollen may induce parthenogenetic development in this species. Realizing that pollen tube growth may be important to the initiation of such development, these results would suggset that sterile pollen did not stimulate parthenogenesis, or if it did, such individuals occurred only at a frequency lower than 1 in 20,000.

Analysis of data derived from the pollen germination trials demonstrated that pollen germination and tube growth are highly resistant to gamma radiation, with no significant differences appearing between irradiated and unirradiated pollen for percent germination until exposures of 100KR to 200KR were reached. These levels are far in excess of exposures required to destroy nuclear viability as judged by the upper limits of seed germination. Pollen tube growth appeared to be somewhat more responsive to discrete increases in radiation exposure than was germination *per se.* Both events could still be quantified at the highest level of irradiation utilized (800KR), but at this point germination averaged only 2.5 percent, and tube length was reduced to 26 percent of the Control. In all likelihood, pollen tube elongation at exposure levels above those at which viable seed is produced is independent of nuclear control, and is a function of metabolic activity in the cytoplasm.

The electron spin resonance sepctroscopy study indicated that the technique may have some application in pollen irradiation programs. It was proposed that if qualitative correlations could be discerned between seed and pollen germination percentages and the characteristic ESR-derived spectra for different pollen irradiation exposures, this method might then offer a rapid means of determining the proper exposures to use in pollen irradiation programs with other species in the genus. The technique might also be extended to other genera, if variables such as chromosome number and nuclear volume could be taken into account.

The "Induced" peak in the irradiated sample spectra occurred in the same region of the spectrum as the weak signal derived from the unirradiated sample. The curve representing the ratio of the Induced peak amplitude to the Control peak amplitude exceeded unity between 18KR and 32KR, in the range of exposures at which pollen nuclear viability terminated. In this study, such a relation-

ship was of course dependent on the particular manufacture spectrometer tubes[3]) which were utilized. The induced signal amplitudes could be related in the same manner to that of other commercially available standards or controls such as DPPH (diphenyl picrazyl hydrazyl), and appropriate relationships established. ESR spectroscopy might thus be utilized to determine the upper limits of pollen nuclear viability for a given species, as well as delimiting the maximum exposures to which the pollen could be subjected and still retain its capacity for fertilization.

At the upper levels of irradiation employed in this study, it was apparent that the amplitude regression was approaching a plateau, perhaps representing a free radical saturation condition in which radical recombination was in equilibrium with the production of new radicals. Pollen germination still averaged about 20 percent at 400KR, so ESR analysis may not prove to be particularly useful for examining radiation effects on pollen at very high levels. In any case, no clearly discernible trends could be ascertained at these levels under the conditions employed in this investigation.

The results of the ESR spectroscopy must be considered as tentative at this point, as the spectra were recorded for only one set of experimental conditions. Further investigation will be needed, especially in the range of exposures from 4KR to 50KR, to more fully define and characterize derived spectra under varying experimental conditions. Of interest would be the study of radical production and decay rates with different pollen moisture contents, and *in vacuo* irradiation and spectrometry. It would seem, judging from these preliminary results, that with some refinement this technique may prove a useful adjunct to the tools presently available for pollen irradiation programs.

## Summary

The radiosensitivity of white spruce *(Picea glauca* [MOENCH] Voss) pollen to gamma radiation was studied through the use of a polycross mating scheme as tested in two plantations in different environments. Radiosensitivity was judged by germination percentages of seed derived from the polycross, electron spin resonance spectroscopy of irradiated pollen, and germination of irradiated pollen *in vitro*. The study was designed to evaluate nuclear and cytoplasmic radiosensitivity, and the possibility of parthenogenesis induction through the use of irradiated pollen.

Regression analyses of seed germination percentages from the irradiated pollen polycross demonstrated a significant decrease in germination as the pollen exposure level increased, with an $LD_{50}$ level occurring at 4KR to 6KR. Extrapolation of the regression line for each plantation suggested that 24KR to 26KR may be a reasonable representation of the upper limit of pollen nuclear viability for the species. An exposure level of 6KR to 8KR is recommended for use in a gametic irradiation breeding program for the genus *Picea*. No seed germination occurred for approximately 20,000 seeds obtained from pollen exposure levels of 50KR to 400KR, and thus no evidence was obtained to indicate parthenogenetic development.

The results of the *in vitro* pollen germination trials indicated an $LD_{50}$ at 300KR to 400KR; pollen tube growth exhibited an $LD_{50}$ level at 400KR to 600KR. These levels are far in excess of exposures required to destroy nuclear viability. In all likelihood, pollen germination and tube elongation at these levels are independent of nuclear control, and are a function of metabolic acitivity in the cytoplasm.

The electron spin resonance spectroscopy yielded results which, although not entirely conclusive, indicate that the technique may have some application in pollen irradiation breeding programs. Further studies are suggested, especially in the range of exposures from 4KR to 50KR, to more fully define and characterize derived spectra under varying experimental conditions.

[3]) General Electric fused quartz.

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# Estimate of Self-Fertility in Coastal Douglas-Fir from Inbreeding Studies

By FRANK SORENSEN[1])

## Introduction

Success of several methods of tree improvement can be influenced by the self-fertility of a species. The purpose of this paper is to present selfing results from Douglas-fir trees in western Oregon and to discuss those results in relation to management of seed orchards, to evaluation of open-pollinated progeny tests, and to development of homozygous lines.

Yield of filled seeds following self-pollination of Douglas-fir is reported to vary among trees from zero to about 20 filled seeds per cone[2]) (ALLEN, 1942; DUFFIELD, 1950; ISTRATOVA, 1964; ORR-EWING, 1954, 1956, 1957 a and b; WHEAT, 1965; SZIKLAI, 1966). One exceptional tree yielded as many filled seeds per cone after self- as after cross-pollination (ORR-EWING, 1957 b). There is also indication that average self-fertility of trees may vary among geographic areas (ORR-EWING, 1957 a).

Initial observations of self-fertility in Douglas-fir were made on isolated trees planted in Europe. These trees had low natural seed yield, and it was first suggested that this was because pollen shed occurred before female strobili on the same tree were receptive (LARSEN, 1937). Later, however, it was found that production of selfed seed was usually low even after controlled self-pollination (ALLEN, 1942; DUFFIELD, 1950). This indicated internal barriers to selfing.

ORR-EWING (1956, 1957 b) investigated this problem by cytologically studying ovule development after self- and cross-pollination. He observed that after selfing, pollen germination, syngamy and proembryo formation proceeded normally, but that embryos almost all collapsed soon after proembryo formation. From this it was concluded that embryo collapse was an inbreeding effect, caused by increased homozygosity of recessive lethal and deleterious genes.

The present investigation was undertaken to enlarge the sample of selfed Douglas-firs, partially because several of the above reports were based on planted trees of unknown or unidentified origin, and partially because all were based on relatively small numbers of trees.

Thirty-five Douglas-fir at five locations in western Oregon were self- and cross-pollinated in one or two of the years 1964, 1965, and 1966.

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[2]) Maximum yield following cross-pollination ranges from about 50 to about 90 filled seeds per cone, depending on cone size, in the coastal form of Douglas-fir.

Seed yields were counted for both types of pollination. Relative self-fertilities were determined for each tree and expressed as the ratio of seed yield following selfing to seed yield following crossing.

Loads of deleterious genes responsible for the observed reduction in seed yield following self-pollination were calculated for each tree.

## Materials and Methods

Trees were selected for self- and cross-pollination in the following five localities on a west-east transect across the central Oregon Coast Ranges and the Cascade Mountains *(Figure 1)*.

| Location | Longitude | Latitude | Elevation |
|---|---|---|---|
| | | | *(feet)* |
| Elk Creek | 123° 45' W | 44° 33' N | 150 |
| Marys Peak | 123° 30' W | 44° 30' N | 3,550 |
| Corvallis | 123° 15' W | 44° 39' N | 450 |
| Lacomb | 122° 42' W | 44° 35' N | 900 |
| Santiam Pass | 121° 48' W | 44° 25' N | 4,400 |

Elk Creek, Marys Peak, Corvallis, and Lacomb plots are in essentially continuous forests which are almost pure Douglas-fir. At Santiam Pass, Douglas-fir is a component of a mixed forest. The five locations bracket the main longitudinal range of coastal Douglas-fir in central Oregon.

Six trees were tested at each location with the exception of Lacomb, where 12 trees were selfed, and Marys Peak, where all cones on one of the six trees were lost to frost. Trees were selected solely on the basis of adequate crops of male and female strobili. Where possible, trees were separated by 200 feet or more, so closely related trees would probably not be sampled. However, the structure of the stands at Marys Peak and Santiam Pass did not permit this. Consequently, some of the tested trees within these two locations may be more closely related than at the other locations.

*Pollination Techniques:*

Female strobili were isolated in pollination bags before opening of floral bud scales. Male strobili were removed from twigs which carried female strobili isolated for cross-pollination; male strobili were left on twigs which bore female strobili isolated for selfing.

Two methods of self-pollination were used. One method, called "self-shake", was to shake the isolation bags covering male and female strobili on two or three different