specific gravity, were compared by pulping and running standard beater evaluations on representative chip samples from single trees from each clone. The results obtained were compared with wood and fiber properties obtained from disk samples and earlier evaluated increment core samples. The trees evaluated were approximately ten years old and the wood was primarily juvenile wood. Because the trees were selected for extremes in specific gravity, considerable variation existed in specific gravity and related fiber morphology. In evaluation of the results, the selection procedure used should be kept in mind.

Simple correlations demonstrated that specific gravity was correlated with the percent summerwood, the percent compression wood, and the wall thickness of the summerwood fibers. Increases in specific gravity were accompanied by increases in tearing strength and reductions in burst and tensile strength. Handsheets made from the high specific gravity trees had lower apparent density indicating the thick-walled summerwood and compression wood fibers were not collapsing with the result that open textured, bulky sheets of low apparent density were produced. Highly significant correlations were obtained between pulp yield and percent lignin ($r = -.98$) and pulp yield and the arithmetic average fiber length ($r = .96$).

Multiple regression analysis revealed that tensile strength was significantly related to the percent summerwood, and the wall thickness of springwood and summerwood tracheids. Tearing strength was related to the above factors and, in addition, was significantly correlated with the diameter of the springwood tracheids. Bursting strength was less influenced by fiber and wood properties and only fiber length was found to be significantly related to bursting strength.

There is considerable evidence in the literature that indicates wood specific gravity is under moderate to strong genetic control. Specific gravity and associated fiber morphology appeared to have considerable influence on pulp and paper properties. The desirability of the use of forest genetic techniques to increase specific gravity of the southern pines is influenced by a number of factors. The importance of increased pulp yield and higher tearing strength *versus* lower apparent density, lower burst and lower tensile strength must be considered. Also worthy of consideration are the possibilities of technologically modifying fiber properties as well as the possible use of silvicultural techniques (fertilization, irrigation, spacing, and length of rotation) to influence specific gravity and fiber morphology.

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Maie Bud and Pollen Radiosensitivity in Selected Conifer Species[1])

By G. R. Stairs and V. Troendle[2])

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Introduction

Mutation induction in forest trees may be accomplished by using ionizing or particulate radiation, chemical mutagens, or by other physical treatments. Definitive comparisons of these various techniques in forest tree materials have not been made and much remains to be learned about the relative advantages of each. Prior to such comparisons, the usefulness of each separate approach must be defined for the broad spectrum of available materials. The study herein reported was conducted to provide data for planning mutation breeding programs in conifers, using gamma radiation of male buds or pollen as the mutagen treatment. It is expected that subsequent breeding studies would provide a basis of comparison to other induced mutation methods.

Reviews of radiation experiments conducted in forest tree material are included in recent publications by Ericksson *et al.* (1966) and Lynn (1967). From these listings it is apparent that the majority of studies thus far have dealt with the irradiation of seeds or other somatic material~Despite the preponderant use of somatic tissues, certain theoretical advantages of gametic irradiation are obvious. In particular, gamete treatment avoids the disadvantages of chimeral cell populations that often result from somatic radiation. The problems of dominance or recessive mutant gene character are not necessarily made easier, although the question of meiotic screening for chromosomal irregularities in individuals following radiation is expected to be more consistent than with chimeral tissues.

The question why one should desire mutation induction in already heterozygous forest tree material ignores much of the biological potential of the technique. A previous discussion of the usefulness of irradiated pollen has been published by Stairs and Mergen (1964). In that article the authors pointed to the possibilities of producing germinable

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but non-viable (nuclear inviable) pollen by proper radiation exposures, for use in parthenogenesis or incompatability studies. Mutation induction to provide material for selection or to serve as marker genes or chromosomes in breeding studies is equally important and obvious. Less obvious is the potential for reselecting among irradiated materials in studies involving canalized development (see WADDINGTON, 1957); this possibility for selecting previously non-variable characters among an irradiated population may become a most important use of mutation techniques.

Material and Methods

Three studies were conducted: (1) the radio-sensitivity of premeiotic male buds in *Pinus nigra* ARNOLD; (2) pollen radio-sensitivity as judged by *in vitro* germination for *Pinus nigra, Pinus resinosa* AIT., *Picea glauca* (MOENCH) VOSS., and *Picea abies* (L.) KARST.; and (3) pollen radiosensitivity for *Picea glauca* as determined in controlled pollination studies.

All radiation exposures were obtained from a Co-60 source of approximately 600 curies. Dosimetry was determined by use of a Victoreen ion chamber, accuracy of exposure is considered to be plus or minus 10 percent.

Material for Study 1 above was obtained from 40-year-old trees growing under arboretum conditions. Branches bearing male flower buds were collected at early meiotic prophase, bound into tight bundles and exposed to the following levels: control, 0.25 kr, 0.5 kr, 1 kr, 1.5 kr, and 2 kr; radiation exposure time was 16-hours. Following irradiation, cytological collections were made twice daily to the microspore quartet stage. Collections were fixed in 3 : 1 alcohol and propionic acid and stained in propionic carmine. Radiation damage was scored by the number of chromosome fragments at Anaphase I and by the amount of chromosome bridging at both Anaphase I and Anaphase II. A total of 300 cells at Anaphase I and 50 cells in Anaphase II were evaluated for each exposure level.

Pollen for the *in vitro* germination studies (Study 2 above) was collected from mature trees at anthesis and placed for storage in glass vials at 4° C with dessicant. Irradiation was conducted at a rate of 300r/minute to provide the following exposure levels: control, 200 kr, 400 kr, 600 kr, and 800 kr. After irradiation the pollen was germinated in glass-distilled water. Evaluation of germinated grains was made at 48-hours; the germination criterion required the pollen tube length to be twice that of the pollen diameter. A total of 2500 grains were counted for each exposure level in three replicated trials and average values were reported for all treatments.

Pollen for the breeding study with *Picea glauca* was collected and exposed as described in Study 2. The exposure levels were: control, .5 kr, 1 kr, 2 kr, 3 kr, and 4 kr. The pollen was irradiated 24 hours prior to its field application on previously isolated female flowers. An unpollinated control was included to determine the effectiveness of the isolation technique. Seed obtained from the pollination trials was stratified and sown in four replicated blocks in a germinator. Conditions during germination were: 16 hours light, 8 hours dark, light temperature 27° C, dark temperature 21° C. Following germination, the seedlings were transferred to a mixture (3 : 1) of sand and peat moss and grown under greenhouse conditions. Non-germinated seed was examined to determine the extent of empty seed in this category.

Results and Discussion

The radiosensitivity of male buds is depicted in *Figure 1 a, b*. A linear increase in fragmentation was found at Anaphase I with values exceeding three fragments per cell at exposure levels of 1 kr. At 2 kr the average values reached six fragments per cell and a great deal of cell death was observed. At Anaphase I the number of chromosome bridges per cell *(Figure 1 b)* increased at a slower rate but still provided a significant linear slope with increasing exposure. Values for Anaphase II were much higher than at Anaphase I and again showed a significant slope with treatment effect. The negative intercept values calculated by the regression are, of course, biologically impossible. They indicate a curvilinear rather than linear relationships in the range between the control and the lowest radiation level studied. In general, these results with male buds were as anticipated on the basis of previous radiation work with other coniferous materials. The rather high radiosensitivity of the *Coniferata* has been related to their large nuclear volume and DNA content (SPARROW and EVANS, 1961) and previous studies have proven this relationship for many conifer species (e. g. PLATT 1960, MERGEN and JOHANSEN 1964, SPARROW and WOODWELL 1962, and SPARROW and SHAIRER 1963). The studies by ERICKSSON *et al.* (1966) suggested a similar sensitivity for male buds in *Larix*, as judged by an elegant technique of mutation evaluation in pollen grains. Greater cytological damage was found for the pine than was reported in a previous study conducted under similar conditions for male buds in oak

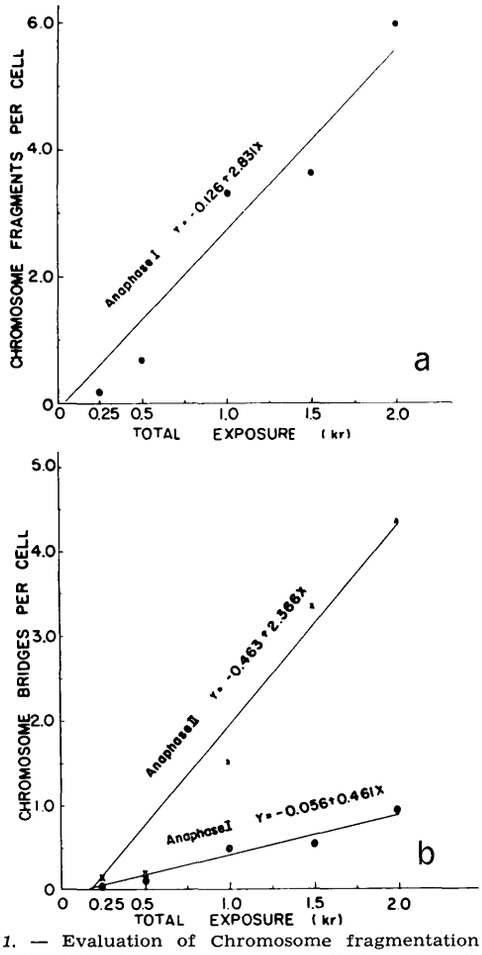

Figure 1. — Evaluation of Chromosome fragmentation (a) and bridging (b) following acute gamma radiation of male buds at early Prophase I.

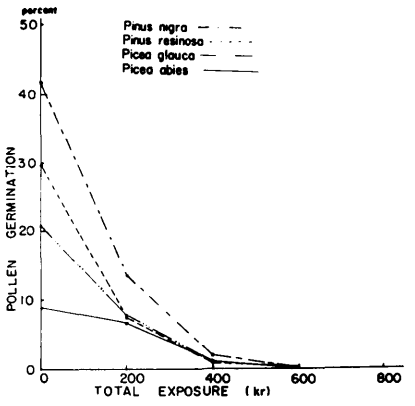

Figure 2. — *In vitro* pollen germination after gamma radiation.

trees (STAIRS, 1964). The established difference between chromosome and nuclear volume in pine and oak would appear to be the significant factor in this comparison. Although meiosis appeared to progress regularly to the quartet stage in the control, none of the buds on the cut branches produced normal pollen. The forcing of conifer buds by this technique is difficult and male bud irradiation for mutation studies may be restricted to situations where portable equipment will allow treatment on the tree. In so doing, additional studies will be needed to correlate actual breeding results with male bud irradiation. Based on the results obtained in this study, radiation levels from control to 500 r are advised for future study, although pollen viability may be obtained at higher levels.

The radiosensitivity of mature pollen is indicated in *Figure 2.* Germination values were slightly lower than is normally expected for conifer pollen; however, the use of only a distilled water media may have been partially responsible for these results. In addition, the requirement for pollen tube length set at 2× the pollen diameter biased the evaluation against partial or weak growth. The control germination values ranged from a high of about 42 percent for *Pinus nigra* and 30 percent for *Pinus resinosa* to a low of 21 percent for *Picea glauca* and 9 percent for *Picea abies.* Despite the variation in initial germination values, response to radiation treatment was similar for all species when based on percent of the control. An approximate LD-50 level was obtained at 200 kr. At 400 kr, germination was less than 3 percent for all species, and lethal exposures were obtained at 600 to 800 kr. The values obtained are aussumed to represent cytopasmic tolerances only, and should not be used to estimate nuclear viability. A similar study of *Pinus densiflora* and *Pinus thunbergii* pollen germination following gamma radiation has been reported by FUJIMOTO and WATANABE (1964). In their work the authors reported that most of the pollen germinated following 75 kr exposures, and some at the 150 kr level. A similar study with oak pollen (STAIRS, 1964) indicated pollen germination after a 400 kr irradiation exposure. Although considerable variation exists between these materials it is obvious that such exposure are several times the expected lethal point for a cell nucleus.

The pollen nucleus viability, as judged by its ability to effect fertilization, was evaluated from controlled pollinations. *Figure 3 a* presents the results of germination trials with seed fertilized by pollen exposed at levels from 0 to 4 kr. The values are based on total seed collected and a calculated regression for this data did not show a significant slope for treatment effect. A similar plot was made based on germination as a percent of filled seed and again the values did not give a significant regression. The latter comparison gave proportionally greater increases for the irradiated material than for the controls although the differences observed were not large. A plot of percent filled seed is shown in *Figure 3 b.* In this comparison a significant treatment effect was observed (F. 05 level) with a calculated linear regression of the formula $Y = 74.68 - 3.72 X$. On the basis of this formula one might predict that a lethal level would be found at about 20 kr. Such a projection would be predicted on the assumption that percent filled seed is a better bioassay of radiation effects than is seed germination based on filled seed. These results are surprisingly high in comparison to the irradiated radiosensitivity of the male buds, but are reasonable in light of the irradiated pollen viability as judged by its germination potential. This differential between cytoplasmic and nucleus sensitivity has been well established for many plants (see BREWBAKER and EMORY, 1962) and it provides the possibility of obtaining germinable but nuclear inviable pollen for use in varied genetic or physiological studies. The values obtained are also high in comparison to the study with this same species reported by RUDOLPH (1965). In that work the author indicated an increase in seed set for treatments at levels up to 0.8 kr.

It is clear from the present study that additional work is necessary to define the nucleus radiosensitivity between the highest level utilized here for the breeding study and the point of lethality as determined by *in vitro* pollen germination studies. At the present time it appears that pollen irradiated under the conditions utilized in this report has about the same range of sensitivity as forest tree

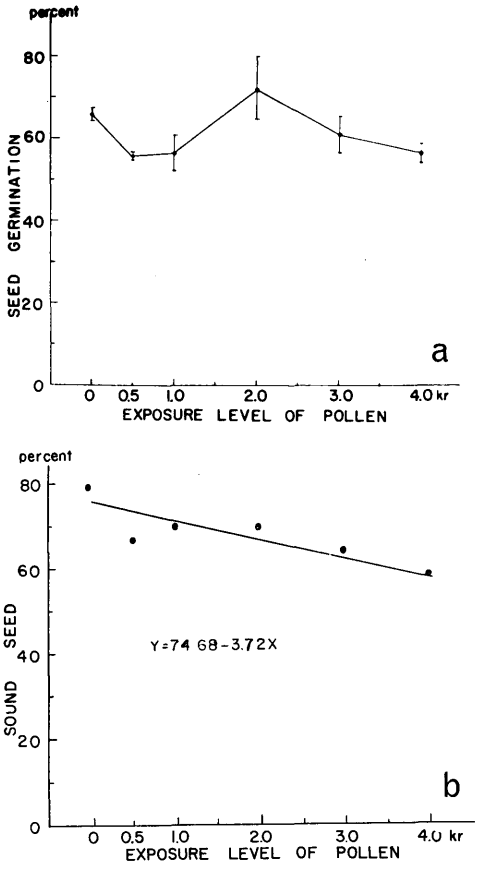

Figure 3. — Seed germination (a) and sound seed (b) percent obtained with seed fertilized by irradiated pollen.

seed. The extremely low moisture content of the pollen undoubtedly plays an important role in such a comparison. Although the limited data indicates that nuclear lethality might be expected at approximately 20 kr, additional study is underway in our laboratory to evaluate a broader range of radiation exposures; the results of that investigation will be published in a later edition of this journal.

Summary

The radiosensitivity of pre-meiotic male buds from *Pinus nigra* was examined in the exposure range of 0 to 2 kr. Exposures above 0.5 kr caused severe cytological damage and it is suggested that treatment of male buds for mutation induction work be restricted to levels below this value. Analysis of irradiated pollen was made for several conifer species. *In vitro* germination was obtained up to 600 kr with an LD-50 at approximately 200 kr. These pollen germination values are assumed to represent cytoplasmic tolerances only. The pollen nucleus sensitivity was examined in a breeding study using *Picea glauca*. Exposures ranged from 0.5 to 4 kr, with a significant increase in empty seed at the higher level, although seed germination based on total filled seed did not show a treatment effect.

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Variation and Taxonomy in Eucalyptus camaldulensis

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Introduction

Eucalyptus camaldulensis, widely called Red Gum is one of the best known of eucalypts both in the wild and as a cultivated plant *(Figure 1)*. In natural occurrence it is confined to the Australian mainland and it does not appear in Tasmania nor is it found in New Guinea where half-a-dozen species of the genus grow naturally and are shared with Australia.

Within the main Australian landmass of some 3,000,000 square miles it is found in all States. In the ecological sense it has a striking feature. It is a species characteristic of watercourses — either along streams, on adjoining levee banks, or on nearby flood plains *(Figure 2)*. In the case of watercourses in the more arid areas in which water flows spasmodically with long periods of no flow, it is often along the sandy bottoms and the margins too.

It is a distinctive and important tree of many rural Australian landscapes and it has some morphological peculiarities which are absent or infrequent in other species of the genus. In many areas it is often the largest tree by far in places where tree growth is difficult and where surrounding vegetation is at the best merely of tall shrubs. As a cultivated plant it is mainly a shelter or ornamental tree in southern Australia but in plantations outside Australia it is of particular importance as one of the most widely planted species, approached in frequency of use only by *E. globulus, E. grandis, E. gomphocephala, E. tereticornis* and *E. viminalis*.

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Because of its sylvicultural prominence the need for closer examination of the variation patterns within the populations commonly assigned to this species for a century has often suggested itself. Such examination has become more readily possible in recent years with rapidly improving communications. In 1963 therefore, preliminary collections of seed and specimens were made from a range of sites and the study was supported by field examination in 1960 of the populations in Western Australia from Williams to the Murchison River and the peculiar and somewhat related situation in the vicinity of Wee Waa in New South Wales which had been examined first in 1956 by Pryor and Johnson who will report the findings soon.

When provenance collections of seed of eucalypts were accelerated, in 1964 by the Australian Forest Research Institute it was obvious that *E. camaldulensis* should be one of the species first to receive attention. Substantial collections of seed were made from a wide range of localities in 1964 and these are at present the basis for tests in several extra-Australian localities. E. Larson (pers. com.), J. F. Lacaze (pers. com.) and Karschon (1967). From these tests and complementary ones in Australia much more detailed information will become available in the future and a great deal more will be known about the species. Nevertheless, with such a wide ranging species more intensive sampling and collection still will be necessary to understand it in the thorough way that some European or North American species are known.

While this study is of limited extent so far as the whole is concerned it has revealed a number of points of interest and it is possible to suggest some further ideas about the