SECOND TECHNICAL SESSION

Chairman: Richard F. West

POTENTIAL USES OF IRRADIATED POLLEN IN FOREST GENETICS[1]

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The use of ionizing radiation as a means of inducing heritable changes in forest trees is becoming increasingly of interest to forest geneticists. Considerable experimental work with forest trees has been accomplished in defining basic tolerances to radiation, and this work now serves as a point of departure for mutation breeding studies.

A brief survey of radiation research throughout the United States indicates activity at many forest research stations. A recent report by Snyder, Grigsby, and Hidalgo (1961) summarized the results of x-irradiation of Southern pine tree seed. At Emory University an intensive study of ecological consequences of ionizing and particulate radiation is continuing under the direction of Dr. R. Platt (see Platt 1960). The U. S. Forest Service is presently planning a radiation source for installation at the Rhinelander Forest Genetics Station (T. Rudolf 1962). Project work supported by the AEC at the Oak Ridge laboratories, Argonne National Laboratory, and Brookhaven National Laboratory has included the study of effects of radiation on forest trees. At the Brookhaven laboratory the cytological investigations by Dr. Arnold Sparrow (Sparrow and Miksche, 1961), and recent ecological studies under the direction of Dr. George Woodwell (Woodwell 1963) have been helpful in defining the tolerance of forest trees to ionizing radiation. Investigations at Yale University by the authors have been concerned with specific tolerances of reproductive structures in pine and oak trees exposed to chronic and acute irradiation (Mergers and Stairs 1962 a, b). In addition to work mentioned here, there are a great number of reported and continuing studies in the United States and abroad concerned with radiation effects on forest trees.

This brief review of work in progress is presented to point out the availability of existing data. At the risk of generalizing it may be noted that the majority of this work has been concerned with seed irradiation, Less work has been accomplished with mature tree irradiation or somatic irradiation of parts thereof; and much less with gametic irradiation. Such an imbalance reflects at least two interdependent factors: (1) seeds are easier to manipulate and withstand broad extremes of physiological variation, and (2) because of the ease of

[1] The work by the authors referred to in this paper was carried out under Contract No. AT(30-1) 2755 AEC, Division of Biology and Medicine, Biology Branch.
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The Brookhaven National Laboratory is operated under the auspices of the U. S. Atomic Energy Commission.

handling, a great store of data has arisen concerning methods of treatment; this in turn has lead to additional studies along something of a path of "lowered resistance". Despite the practical advantages of seed irradiation, there is no a priori genetic reason to discriminate against gametic irradiation as a means of mutation induction. Admittedly definitive treatment of the macrospore is not recommended for the uninitiated; however, irradiation of microsporogenesis or of microspores is more feasible. In addition to the potential for mutation induction there is the possibility of influencing cross incompatibility mechanisms. Davies and Wall (1961) were able to overcome the incompatibility of the cross Brassica oleracea x Brassica nigra by irradiating either the male or female parent. The use of radiation to induce self-fertility mutations in fruit trees by treatment of dormant male buds has been discussed by Lewis (1949) and Lewis and Crowe (1954). In the latter reference the authors obtained about 10 per cent self fertile seedlings by using pollen from male buds irradiated prior to meiosis. Present work by the authors allows a prediction of tolerance levels for oak and pine pollen irradiation (Mergen and Johansen, 1963; Stairs, 1963). The relation of nuclear volume to radiosensitivity reported by Sparrow (1961) suggests that our data may be projected to other forest trees of similar nuclear volume.

Use of irradiated pollen in forest tree breeding work may be cateogrized in two general areas: (1) treatment at relatively low levels of radiation designed to induce gene, chromosome, or cytoplasmic changes without destroying nuclear viability, and (2) irradiation at higher levels designed to inactivate the generative nucleus but to still allow pollen tube growth as an expression of cytoplasmic function, The first of these two methods may be used in pollinating with irradiated pollen to obtain mutations among the progeny produced. Mutations would be observed in the Mendelian sense among the F_1 generation if dominant, or at later generations if recessive. In terms of quantitative inheritance of such complex traits as components of total growth the consequence of irradiation will be more complex. A "shift" of the variance pattern for a particular trait resulting from quantitative changes (mutation) at many different loci may result. Within such an irradiated population a search will be necessary to find the fortuitous genetic change that results in desirable phenotypic expression. Because we are dealing with traits that are controlled by quantitative inheritance in most of the growth characteristics of forest trees the potential for mutation induction in a particular sequence is greatly increased. However, the total effect of a single mutational event may be small. It is in this particular area of research that additional data are needed. The authors hope that studies will be undertaken by others in the field to aid in evaluating the mutation spectrum obtained by use of irradiated pollen, Only by dealing with large populations, using different species and levels of radiation exposure, will the real value of this breeding tool be properly evaluated.

The second suggested use of gametic irradiation entails use of genetically inert but physiologically active pollen. Such a selective situation may be obtained by taking advantage of the differential radiosensitivity between the nucleus and the cytoplasm. In reviewing work on the irradiation of angiosperm pollen, Brewbaker and Emery (1962) reported inhibition of germination and pollen tube growth only after exposure to massive doses, with the LD50 values ranging up to 550,000r. By contrast, pollen tube divisions were restricted to an average dose of 250r. at the LD50 level. Thus it is possible to irradiate pollen at very high levels which will probably inactivate the nucleus but will allow normal or near normal germination and tube growth.

In studies with pine and oak the authors have exposed pollen from Pinus rigida, Pinus strobus and Quercus alba to radiation levels up to 30,000r. After exposure to 300,000r. up to 70 per cent of the pollen for all three species germinated and no significant reduction was found in pollen tube lengths. However, the germination results obtained were more variable after irradiation than the controls. Forking, balooning, and other growth phenomena were observed at all levels, including the controls and there was no increase in forking as a result of radiation. After the germination tests, some of the irradiated pollen of Pinus rigida was stored in a desiccator under refrigeration, and after a storage period of 10 months, the viability as evaluated by germination in 2.5 percent sucrose was 61 per cent for pollen exposed to 300,000r.

Genetically inactive but germinable pollen may be used as a cytological tool in several ways. Among these are the potential stimulation of partheno genesis, including monoploid production; overcoming minor cross-incompatibilities, and physiological studies of incompatibility in forest trees. Present studies are in progress by the authors using pollen of this type to facilitate crossing in spruce and pine where previous studies have shown inter species incompatibility. The method involves mixing compatible but genetically inactive pollen with normal pollen of the incompatible species and applying the mixture to receptive strobili of the desired mother tree. In addition to studies involving crossing patterns, attempts to stimulate parthenogenesis in spruce and pine are underway at Yale and at the College of Forestry at Syracuse.

In conclusion it may be restated that the background of information concerning the radiosensitivity of forest trees is broadening rapidly. A logical projection of this information in terms of forest tree improvement work will be to initiate and evaluate the results of breeding with irradiated pollen. Facilities such as X-ray machines are readily available, the College of Forestry at Syracuse has a $C0^{60}$ source which may be used on a cooperative basis, and many of the AEC sponsored laboratories will perform service irradiations for bona-fide research projects. The need is for many researchers to join in a cooperative effort to evaluate properly the role of mutation breeding in forest trees. At least one method of doing this will be by use of irradiated pollen.

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