CHRONIC AND ACUTE RADIATION OF MICROSPOROGENESIS AND MATURE POLLEN IN QUERCUS

by

G. R. Stairs***

Yale University, School of Forestry, New Haven, Conn.
Biology Department, Brookhaven National Laboratory, Upton, N.Y.***

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission: A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or adequacy of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or B. Assumes any liability with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor preserves, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

* Work performed under Contract No. AT (30-1) 2755, Atomic Energy Commission, Division of Biology and Medicine, Biology Branch; awarded to Dr. François Mergen, Yale University, School of Forestry.

** Present address: State University of New York, College of Forestry, at Syracuse University, Syracuse, New York.

*** This laboratory is operated under the auspices of the U.S. Atomic Energy Commission.

The article is part of a study on radiation effects in oaks carried out by the author in partial fulfillment of requirements for the Ph.D. degree at Yale University.
DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.
DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.
CHRONIC AND ACUTE GAMMA RADIATION OF MICROSPOROGENESIS AND MATURE POLLEN IN QUERCUS

INTRODUCTION

The sensitivity of pollen production is a major component of sexual reproductive ability in a forest exposed to ionizing radiation. Ample evidence has accrued in the literature showing that the meiotic process is one of the most sensitive stages in the plant cycle (see Sparrow et al., 1952; Sax and Swanson, 1941). Within most forest tree populations pollen is wind distributed and represents the pathway for gene flow throughout sympatric breeding systems. Exposure to ionizing radiation can produce an effect on the population ranging from genetic change (e.g., gene mutation, chromosome aberration) capable of dissemination in a breeding system, to pollen abortion and interference with normal seed production.

With the present day increase in the use of ionizing radiation it becomes desirable to define the cytological response from long term (chronic) and short term (acute) exposures to radiation. In addition to the ecological significance such information is also of fundamental value to the forest geneticist interested in utilizing induced mutation for breeding work. Most of the genetic characters of commercial interest to the field of forestry are quantitative in nature, and with the long breeding cycles found in forest trees genetic improvement by recombination is slow. The use of induced variation offers one potential method to accelerate selection of desired
individuals. Some data are available on the radiosensitivity of oak tree seed (Heaslip, 1959) but little has been reported on irradiation of microsporogenesis or mature pollen. The cumulative effects of low level chronic radiation on an oak-pine forest have been studied by Mergen and Stairs (1962). The production of pollen was more sensitive in pine than oak, and both genera showed a cumulative aberration effect after several years of irradiation. Bogdanov (1948) reported no persistent changes when irradiated poplar pollen was utilized in breeding trials. 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 percent self fertile seedlings by using pollen from male buds irradiated prior to meiosis.

While the majority of induced mutations in plant breeding have been obtained by irradiating seed there is no a priori reason against treatment of male flower buds or mature pollen as a means of inducing mutation. The possible efficacy of gametic irradiation was discussed as early as 1930 by Stadler but little subsequent work has been accomplished in this area.

The following experimental work was conducted to determine the tolerance of pollen production in *Quercus* to gamma radiation. The effects of chronic and acute irradiation of microsporogenesis and mature pollen were investigated; where possible species from both sub-genera within the genus were utilized.
MATERIAL AND METHODS

Chronic Radiation

Male floral phenology and microsporogenesis in Quercus were investigated under conditions of whole tree irradiation. The species utilized were Quercus alba Linna., Quercus ilicifolia Wang., and Quercus coccinea Muench. The first of these species is from the Lepidobalanus subgenus while the latter two are assigned to the Erythrobalanus group.

The studies were conducted on two separate sites at the Brookhaven National Laboratory; the two locations will be designated herein as the Gamma Field and the Gamma Forest. The Gamma Field was installed in 1951 by the Brookhaven Biology Department to provide a source of gamma radiation for irradiating plants under field conditions. A mixed oak-pine forest surrounds the Gamma Field; because the forest edge began approximately 70 meters from the source only low level radiation reached the trees. Actual dosimetry has varied with changes in source size; during this investigation the area studied received an exposure range of 1 to 15 r/20 hour day. The field was exposed to irradiation only during the growing season for the first 8 years of operation; since 1959, the exposure has been continued throughout each year. For further operational details the reader is referred to Sparrow (1960). Figure 1A depicts the Gamma Field area under investigation in relation to the Co60 source.

The Gamma Forest source was placed in operation on 22 November 1961, and has been maintained on a 20 hour day exposure rate since that time. The radiation source of 9500 curies
Cs$^{137}$ was placed in a natural oak-pine forest by Brookhaven personnel to evaluate chronic radiation effects on a complete ecological system. The details of planning and source operation have been reported by Sparrow and Woodwell (1962). Figure 1B shows an aerial view of the Gamma Forest taken in July 1962; the source location is indicated within a circle of approximately 45 meters radius.

Floral phenological observations were made in 1961 in the Gamma Field and in 1962, in both the Gamma Field and Gamma Forest. At the time of pollen maturity catkins were collected from varying dose levels at both locations. To determine pollen abortion rate, selected anthers from each level examined were fixed in Newcomer's solution and evaluated after staining with propionic carmine. Each exposure level was scored by counting the abortion rate in a total of 500 grains on each of four slides. In addition to pollen abortion frequency, the mature pollen collected was evaluated for germination percent and pollen tube length. Pollen for germination was collected at anthesis and stored at 4°C; for germination trials an aqueous media of double distilled water containing 20 ppm boron was utilized. Germination proceeded in a hanging drop of culture solution suspended over a Van Tiegham cell at an incubation temperature of 30°C; a germinated grain was considered as one with a tube length 2x the pollen cell diameter at 24 hours. Pollen tube length was measured
after 24 hours on 20 grains of each culture cell.

Acute Irradiation - Microsporogenesis and Mature Pollen

Representative species from the two subgenera utilized in the chronic radiation study were evaluated under conditions of acute gamma irradiation. The two species used were *Quercus alba* and *Quercus coccinea*. Previous investigation had shown that viable pollen was produced when cut branches bearing dormant male buds were placed in water under favorable conditions of temperature and relative humidity. Utilizing this method for radiation treatment, branches bearing male buds were cut and bound into tight bundles, with the lower stem portion maintained in water from the time of cutting until anthesis. Two exposure conditions were studied: (1) a 16-hour acute period utilizing a Co$^{60}$ source of approximately 3200 curies with total exposure ranging from 1000 to 8000 r, and (2) a semi-acute study utilizing a Co$^{60}$ source of approximately 15 curies operated on a 20-hour day exposure schedule. The latter source was located in a greenhouse at the Brookhaven National Laboratory; exposure levels ranged from 100 to 1000 r/20 hour day. A total of 5 acute irradiation series were made: three using *Quercus alba* irradiated at the pollen mother cell stage (PMC), prophase I (PI), and anaphase II (AII); and two with material from *Quercus coccinea* irradiated at PI and AII. A single series was investigated in the semi-acute study using material of
**Quercus alba.** Following the radiation treatment branches of Group 1 (16 hour acute exposures) were placed in a controlled greenhouse environment (25°C days, 19°C nights). Group 2 (semi-acute 20 hour day exposures) was allowed to develop under continuing radiation in the Gamma Greenhouse.

The evaluation of acute radiation effect was made on the basis of cytological damage to chromosomes, pollen abortion, pollen germination and pollen tube length. Collections for cytological evaluation were fixed and stored in Newcomer's solution; a mordant solution of propionic carmine gave good staining results. Chromosome aberration was scored at late anaphase I and is based on the number of fragments observed per 100 cells. The chromosomes in *Quercus* are small (ca. 1 to 4.5 microns long) and one cannot readily differentiate chromatid structure with a light microscope; therefore, identification of bridging types was not attempted. In assessing total fragmentation each chromosome bridge was assigned a value of one.

Collection, storage, and evaluation of pollen was conducted in a manner similar to that described for the chronic study. Observation of catkin morphology was made at the time of pollen shedding.

**Acute Irradiation - Pollen**

Pollen was collected at the time of natural shedding and stored in glass vials at 4°C. For treatment the pollen was exposed to acute irradiation (16 hours) from a Co$^{60}$
source of approximately 3200 curies to provide exposures of 50 to 300kr. Following irradiation the pollen was sown in a hanging drop of double distilled H2O solution containing 20 ppm boron and was germinated at a temperature of 30°C. Tube lengths were measured on 20 germinating grains from each treatment level after 24 hours.

**CHRONIC IRRADIATION RESULTS**

**Floral Phenology and Morphology**

Floral phenology and pollen shedding in *Quercus ilicifolia* and *Quercus coccinea* were initiated about 14 days before that of *Quercus alba*. A delay in flowering was observed which was positively correlated with exposure level. This response was clinal in both the Gamma Field and in the Gamma Forest. Male buds from the Gamma Field are illustrated in Figure 2, and from the Gamma Forest in Figure 3. In Figure 2A the condition of the male buds of *Quercus alba* is illustrated five days before pollen started to dehisce in the controls. The delay of male flowers from trees exposed to 8 to 12r/20 hour day is quite pronounced. At the time of pollen shedding, on May 26 (Figure 2B), the delay was not as great but pollen shedding was delayed by approximately one week. The irradiated catkins were shorter than the controls at pollen shedding and did not reach full vegetative maturity.

The condition of *Quercus ilicifolia* and *Quercus coccinea* on May 10 is illustrated in Figure 2C and 2D.
respectively. The maturation of male flowers and pollen shedding followed the pattern previously described for *Quercus alba*.

While a similar retardation of phenology was observed in the Gamma Forest, it occurred at a higher radiation level than in the Gamma Field. The difference in exposure time during the formation and growth of buds for the two areas may account in part for this difference. Figure 3A and 3B depicts the buds of *Quercus ilicifolia* and *Quercus coccinea* on May 5, while Figure 3C indicates the condition at pollen shedding in *Quercus ilicifolia* (May 10) and *Quercus alba* (May 26). At the higher radiation levels the vegetative development was greatly reduced (Figure 3C-D) with pollen being shed from catkins that were approximately 1/5 the length of normal structures.

**Pollen Abortion, Germination, and Tube Length**

Pollen collections from the Gamma Field were obtained from levels ranging between 1 and 15 r/20 hour day. Pollen abortion showed a positive correlation with increasing radiation level and the data for each species expressed a linear function when plotted over daily exposure rates. The data for the three species were kept separate for the analysis, and tests of significance between the regression lines of the transformed data (arcsin) showed that there was no significant difference between the three species in either the Y intercept (a), or in the slope (b). Therefore, a common slope was calculated for the three species,
and this regression is illustrated in Figure 4. Control values are indicated on the graph ordinate and the total exposures received by the mother trees are shown on the abscissa. The values did not deviate greatly from the calculated regression with the exception of the pollen from a single individual of *Quercus alba*. This tree was exposed to approximately 9r/20 hour day and had a considerably higher percentage of aborted grains. This particular tree also showed greater morphological aberrations and appeared to be in poor physiological condition.

In the Gamma Forest pollen was evaluated from trees at the edge of the survival zone. (Ca. 45r/day) and out to a level of 3.7r/20 hour day. Pollen abortion showed a positive linear relationship with increasing exposure level. However, the abortion rates which occurred at the higher levels in the Gamma Forest were similar to those observed at lower levels in the Gamma Field. As previously pointed out the two areas had different past histories in relation to radiation exposure. As in the Gamma Field data there was no significant difference in pollen abortion between species and a common regression line was calculated as shown in Figure 5. The total accumulated dosage from inception of the source in November 1961, to the time of analysis is indicated on the graph.

Germination trials with control pollen of *Quercus* indicated wide variation between replications. Because
the results are extremely sensitive to environment, both before and during *in vitro* germination, the reproducibility is somewhat doubtful. This is especially true in regard to comparisons between different areas or treatments; therefore, the germination results reported are qualitative. In the chronic studies pollen germination was obtained from all of the radiation levels examined. The results were variable, and showed no direct relation to radiation when based on germination as a percent of normal grains. In several trials, germination of pollen collected at the higher levels from both areas was equal to the control (based on percent of normal grains). Nevertheless, there was considerably more variation in the irradiated lots compared to the controls. The grains which did germinate produced normal pollen tubes with no consistent reduction in tube length or rate of growth. The tube sizes from the irradiated pollen were more variable, and were in some instances longer than those of the control.

**ACUTE IRRADIATION RESULTS**

*Floral Phenology and Morphology*

Material exposed to acute radiation did not reach full vegetative maturity at the time of pollen shedding. There was also a delay in pollen shedding within the irradiated material, however, this delay was less (2-3 days) than that observed in the chronically irradiated group. The acute exposure necessary to inhibit bud enlargement
varied within a species dependant on the developmental stage at which radiation was received but no distinct difference between species was observed. In the five experiments conducted both species showed complete inhibition of male buds at an exposure of 4kr when irradiated at the PMC or early PI stage. When buds were exposed during meiosis proceeding from AII to the quartet stage the lethal point for vegetative growth was extended to Ca. 6kr.

Cytological Evaluation

The rate of chromosomal aberration for an acute series irradiated during early prophase I of the first meiotic division is presented in Figure 6A. Calculated regression lines for number of fragments per 100 cells and that for number of normal cells are shown. Both regressions are significant at the 1 percent level, and are essentially reciprocal. At the highest level of radiation there were 19 normal cells observed per 100 cells examined while the frequency of aberration at this level was 3.2 fragments per damaged cell.

In addition to aberration at anaphase I, additional bridging and fragmentation were evident at anaphase II. Abnormalities observed at anaphase II, while undoubtedly contributing to pollen abortion rate, were not included in the cytological scoring. Examples of the fragmentation and bridging observed are illustrated in Figure 7B-F, along with a normal metaphase I (Figure 7A) of the first meiotic division. Representative pollen from control and
irradiated buds is seen in Figure 7G-M.

The results of a semi-acute (100-hour) exposure are shown in Figure 6B. In this graph the cytological scoring from a series irradiated for five days in the Gamma Green-house are presented in a manner similar to Figure 6A. The number of chromosome fragments/100 cells ranged from 40 at 0.5kr to 380 at an exposure of 5kr.

The intercept values (a) for both the acute and semi-acute regressions are negative. Although the regressions are linear over the dosage responses studied, there may be a non-linear portion of the curve below the levels studied which would account for this slight discrepancy.

The number of bridges observed at AI provides a comparison of the more complex chromosomal breaks induced in the 16 and 100 hour exposures (Figure 8A). The 16-hour irradiation shows a curvilinear response with a greater increase at higher levels as compared to the 100-hour exposure. Due to different chromosome stages in the two treatments during irradiation a complex of chromosome, chromatid, or sub-chromatid aberrations may have been induced. Evans (1962) reviewed the subject of chromosomal aberrations and pointed out that the fundamental difference between these three types is the unit of breakage or exchange. Because the type of bridging was not defined the data indicates a summation of all types of observable bridges.

The relationship between chromosome breakage and pollen
abortion for a 16-hour acute treatment is shown by the two regression lines in Figure 8B. Both regressions are significant at the .01 level; a statistical analysis of the two groups shows no significant variation in slope (b) between the two. However, there is a significant (.05 level) difference in intercept values (a).

The results of five acute (16-hour) radiation treatments for two species of oak are plotted in Figure 9. For Quercus alba the microspore stages irradiated were: PMC, PI, AII. In Quercus coccinea only the latter two stages (PI and AII) were examined. An analysis of variance for the five groups indicated no significant variation between species, or between series irradiated at PI and AII. There was a consistently lower abortion rate in the group irradiated at the PMC stage and this difference is significant at the .05 level. Therefore, two regression lines were calculated; one for the group irradiated at the PMC stage, and a second for the remaining four groups. In the graph the broken lines extrapolate from the sub-lethal levels for each division stage irradiated. The variation observed in pollen abortion under control conditions is shown on the graph ordinate (Figure 9).

Pollen Germination and Tube Length

The variable results of in vitro pollen germination and tube length previously discussed in relation to chronically irradiated pollen were also found in acute irradiated material. However, by collecting pollen as the
anther matured, more consistent results were obtained. This "fresh" pollen was removed from the anther and placed directly in the germination media and evaluated 24 hours later. The variation within germination trials showed a significant increase at the higher radiation levels, as total germination decreased. In all cases pollen germination was evaluated as a percent of normally appearing grains. Figure 10 shows a bar graph of the mean pollen germination and tube lengths at 24 hours in replicated trials with pollen that had been irradiated at prophase during the first meiotic division. The germination rate does not decrease significantly until a level of 4kr is reached. At this level, pollen tube growth is slowed and does not exceed the average diameter of the pollen grain. Thus germination at 4kr is only spurious after 24 hours, although after 48 hours the tubes had grown to a length of 2x diameter of the grain and represented germination as previously defined. Therefore, reduced growth of pollen tubes at higher levels appeared to be due to a slower growth rate at initial stages rather than to an absolute size limitation. In terms of total growth and growth rate the irradiated pollen did not differ significantly from the controls up to and including a microspore exposure of 3kr. The greater average tube length observed at an exposure of 1kr was not a significant stimulation effect.

**Mature Pollen Irradiation**

Pollen was collected at anthesis from control trees
and exposed to radiation at levels from 50 to 300 kr. Evaluation of germination and tube lengths showed the irradiated pollen to be extremely resistant to radiation. In a total of 3 replicated trials no correlation could be found between radiation level and germinative ability up to exposures of 100 kr. At 200 kr the germination results were much more variable than the controls, although some trials did as well as the control group. With a total exposure of 300 kr the germination varied from 20 to 70 percent of the control group. Pollen tube growth was also variable; with no apparent reduction up to a total exposure of 200 kr. At 200 kr the tube growth was slightly suppressed and at 300 kr was only 60 percent of the control length. In as much as these levels are far in excess of the normal cytological tolerance encountered in other stages of the life cycle, it would appear that the germination observed resulted from cytoplasmically controlled metabolism. The variable sensitivity of different cell portions has been examined by use of microbeam irradiation (see e.g. Zirkle, 1957). Evans and Sparrow (1961), reviewed nuclear factors affecting radiosensitivity and pointed out that the nucleus is much more sensitive to radiation than the cytoplasm. Therefore, while further work is necessary it would seem that pollen irradiated at levels of 300 kr was genetically sterile. Growth of the pollen tube could result from preformed endogenous substrate and metabolic activity in the cytoplasm which is highly resistant to radiation.
DISCUSSION

The effect of cumulative chronic radiation on pollen production in the two radiation fields may be a combination of genetic and physiological damage. In the Gamma Field, radiation has been a component of the environment for a period of years, and damage at the cellular level resulted in a decrease in gross meristematic area per tree. This decrease is reflected by sparse and abnormal foliage, and a lowered apparent vigor. Concomitant with radiation damage the susceptibility of the trees to biotic stress such as insects and disease is increased. The sum of all such constituents as they act through the physiology of microsporogenesis is reflected in the pollen abortion values. Mericle et al. (1963) reported on the abnormal morphological expression of two oak trees growing in the Gamma Field and the reader is referred to this paper for a discussion of somatic effects from chronic low level radiation. Trees in the Gamma Forest had received radiation for only 5 months at the time of this study and evaluation of radiation damage is not as complex as in the Gamma Field.

Direct separation of genetic and physiological effects is not possible in terms of the analysis made for either area. In addition to chromosomal damage by radiation, the destruction of endogenous growth substances may play a major role in determining viable pollen production. Reports by Skoog (1935) and by Gordon (1957) have shown the relative sensitivity of auxin metabolism to radiation.
Sparrow and Schairer (1958), reported that application of TIBA to Nicotiana hybrids gave results similar to radiation treatment. Although auxin metabolism is one of the most radiosensitive reactions found to date, other biochemical lesions have been reported from radiation experiments. Creasey and Stocken (1959), demonstrated that nuclear phosphorylation is a highly radiosensitive process. Inhibition of this nuclear energy source may be particularly important in cells with a large nuclear/cytoplasm ratio (Kelly, 1961). Other factors of biochemical radiosensitivity include DNA synthesis (for review see Lajtha, 1960), RNA synthesis, and protein synthesis (see Kelly, 1961). The delay in bud breaking observed for both of the chronic radiation areas may be a response to altered cell chemistry, either in situ at meristematic areas, or in synthesis at the molecular level. For further discussion of biochemical radiosensitivity the reader is referred to several recent reviews: Ord and Stocken, 1961; Errera, 1959; and Bacq and Alexander, 1961.

Sparrow et al. (1961) have shown the total accumulation of radiation effects by the cell nucleus to be a major factor in plant radiosensitivity. Data obtained in the study with oaks showed no significant variation between species; all three species investigated had similar nuclear volumes and the results are thus in accord with the nuclear volume hypothesis. It has also been demonstrated that the length of the cell cycle during exposure becomes important in chronic radiation studies (Sparrow and Evans, 1961). These references suggest
that a comparison of radiation accumulation in the Gamma Field and Gamma Forest during the cell cycles prior to pollen shedding would be informative.

The male flowers in *Quercus* are formed during the previous growing season; therefore, in the Gamma Field they were irradiated during formation while those in the Gamma Forest were formed prior to radiation exposure. The exact number of cell cycles responsible for formation and differentiation of primordia was not established. However, by assuming that the primordia cells and their direct descendants are susceptible to radiation from an arbitrary date of July 1, the total exposure received by the population responsible for microsporogenesis may be calculated. The following example is extrapolated from the regression lines in Figure 4. With exposure rates of 19r/day for the Gamma Field and 45r/day in the Gamma Forest pollen abortion values of 30 and 29 percent respectively are obtained. The total current accumulation for these levels is 4700r in the Gamma Field and 6600r in the Gamma Forest. Comparing these values to the total exposure of Ca. 25000r for the mother tree in the Gamma Field example it would appear that the abortion rates observed are more closely related to annual exposure than to accumulated low level exposure over a number of years. Nevertheless, the larger values obtained at a particular level in the Gamma Field show an effect of accumulated radiation.

The results indicate that the ability to produce
germinable pollen is comensurate with vegetative survival under conditions of chronic radiation. In the Gamma Forest pollen abortion was only about 20 percent at the sub-lethal level (45r/day). Trees in the Gamma Field were still producing viable pollen following 11 years of radiation giving a total accumulated exposure of over 25000r. Since the un aborted pollen that was produced gave near normal germination and tube growth, the propagation of induced mutations from the irradiated trees seems quite likely. In this manner the irradiated areas could function as points of centrifugal evolution in regard to the surrounding population. The actual detection and quantification of mutational events will require future long term evaluation of the R₁ and subsequent generations.

Acute irradiation of microsporogenesis yielded much higher aberration rates than was found in chronic studies at comparable total exposures. Results obtained were expected in view of the different cell cycle lengths; the acute and semi-acute series received radiation during a single cell cycle, while the chronic exposure was distributed over several cell cycles at a lower rate and total accumulation per cell. Therefore, while the results obtained in the acute, semi-acute, and chronic studies may be compared, the factors contributing to these results are not directly comparable.

Despite the differences in exposure conditions and radiosensitivity of the acute and semi-acute irradiations, the data suggest that under conditions examined treatment at the lower exposure rate results in a cytological effect
similar to a comparable total accumulation delivered at a higher rate. The efficiency of acute versus semi-acute or chronic radiation as a means of inducing mutations has received only cursory investigation. Russell et al. (1958) reported chronic gamma irradiation to be mutagenically less effective than acute x-radiation. Although in a later paper (Russell et al., 1959) it was shown that a fractionated dosage gave a higher mutation rate than a single acute dose. While additional studies are needed it seems that in utilizing these methods for mutation breeding the use of a smaller source would be less costly and still yield satisfactory results. In using either acute or semi-acute irradiation for mutation induction it is suggested that the male flower buds of oak be irradiated in the range of 1 to 4kr total accumulation.

Comparison of data for pollen abortion and chromosomal aberration has shown the parallel nature of the two analytical methods. Estimates of radiation effect are more simply made by using pollen abortion and the method may be recommended for species which are difficult to evaluate cytologically. In the acute irradiations pollen abortion values are not linear to the point of lethality. The lack of direct linearity between pollen abortion and lethal levels may be complexed on two levels: (1) the cell populations when irradiated are not completely homogeneous in stage of division, and (2) the previously discussed physiological damage at the cytoplasmic level or in the
transfer system between nucleus and cytoplasm, may be sufficient to decrease or prevent cell division from being initiated.

Irradiation of mature oak pollen has shown a radio-resistance typical of that encountered in other angiosperms. Brewbaker and Emery (1962), have obtained an LD50 level of 250kr when averaging values reported in the literature for mature pollen, versus an average of 250r for pollen tube divisions. The amount of radiation necessary to inhibit germination of pollen is far in excess of normal nuclear tolerance and pollen irradiated at these levels is probably sterile. Therefore, in utilizing irradiated pollen for mutation breeding work the range of sensitivity previously suggested for male flower buds (1-4kr) may be most appropriate. Pollen which had been treated at high levels could be used in experiments where genetically inert tube growth would be desirable. The attempted induction of parthenogenesis would be one example; another would be the use of sterile but germinable pollen as a stimulator to otherwise incompatible crosses.

SUMMARY

The production of pollen under conditions of chronic gamma irradiation was investigated for three oak species. Two chronically irradiated areas were studied: (1) a low level (1 to 15r/day) area where trees had received varying amounts of radiation over a period of 11 years, and (2) a
second area receiving gamma radiation for about 5 months previous to the investigation. In the latter study dose levels ranged from lethal (45r/day) to a region of no detectable effect. In both areas pollen abortion showed a significant increase with increasing radiation exposure, although germinable pollen was produced at all survival levels examined. The germinating pollen tube length did not show a significant decrease in the irradiated material examined. In addition to cytological effects there was a marked delay in floral phenology for both areas.

Acute irradiation of male flower buds at different stages of meiosis, and of mature pollen were reported. The radiosensitivity of microsporogenesis was evaluated by cytological scoring at anaphase I, and by pollen abortion, germination, and tube length. Both the number of chromosome fragments/100 cells scored at anaphase I and pollen abortion showed a linear increase with an increase in radiation exposure. Pollen germination and tube length were less effected by radiation (based on a percent of unaborted grains).

It was suggested that a range of 1kr to 4kr will be appropriate for irradiating male flower buds of oak to be utilized in a mutation breeding program. Contingent upon additional studies the range of radiation recomended for flower buds is also suggested for the induction of mutations in pollen.
Pollen was found to be highly resistant to radiation when evaluated by germination and tube growth studies. No effect was found with irradiation of 100kr; at 300kr both germination and tube lengths were depressed. At these levels it is probable that germination is an expression of cytoplasmic growth and not of nuclear viability.

No significant difference was found between responses of the two species for either chronic or acute irradiation.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Francois Mergen for his valuable help in the study. Special thanks are also due to Dr. A.H. Sparrow, Dr. G.M. Woodwell, and Mr. L.A. Schairer for their co-operation; and to various members of the Health Physics Department for their help with dosimetry. Technical assistance by Mr. A. Petracco is also gratefully acknowledged.
LITERATURE CITED


FIGURE 1. Aerial view of the Gamma Field (A) and of the Gamma Forest (B). The location and type of source are indicated in each photo. The photo in (A) was taken during the dormant season, that of the Gamma Forest (B) was taken during early summer.
FIGURE 2. Effect of chronic radiation on phenology of male flowers in the Gamma Field. (A) Quercus alba collected on May 21, 1962, left to right, upper; control, 5.2r, 6.0r lower; 10.5r, 11.5r, 12.0r (B) Quercus alba collected on May 26, 1962, left to right, upper; control, 3.4r, 5.2r lower; 6.0r, 9.4r, 10.5r, 11.5r. (C) Quercus ilicifolia on May 10, 1962, left to right; control, 9.0r 13.5r. (D) Quercus coccinea on May 10, 1962, left to right; control, 7.4r, 11.5r.

Scale is in mm.

FIGURE 3. Effect of chronic radiation on phenology of male flowers in the Gamma Forest. (A) Quercus ilicifolia collected May 5, left to right; control, 16r, 45r, 180r. (B) Quercus coccinea on May 5, left to right; control, 16r, 45r, 180r. (C) Quercus ilicifolia collected on May 10, left to right; control, 16r, 45r, 84r. (D) Quercus alba on May 26, left to right; upper; control, 1r, 3r, lower; 7r, 16r, 45r, 84r.
FIGURE 4. Effect of chronic gamma radiation on pollen abortion in the Gamma Field. The calculated regression is based on an arcsin conversion of abortion percentages, plotted over exposure rates per 20 hour day. The total accumulated exposure of the mother trees is also indicated.

FIGURE 5. Effect of chronic gamma radiation on pollen abortion in the Gamma Forest. The calculated regression is based on an arcsin conversion of abortion percentages, plotted over exposure rates per 20 hour day. The total accumulated exposure of the mother trees is also indicated.
**FIGURE 4**

Data points for pollen abortion with exposure rate and accumulated exposure.

**FIGURE 5**

Data points for pollen abortion with exposure rate and accumulated exposure.
FIGURE 6. The evaluation of chromosome aberrations resulting from gamma irradiation during microsporogenesis. (A) results of a 16-hour exposure of male buds during PPI. (B) results of a 100-hour exposure irradiated from PMC at AI during meiosis. The total exposure and exposure rate are given.
FIGURE 6A

Fragments / 100 cells

Y = -15.29 + 68.18X F=93.92 (1%)

Normal division figures x

Y = 64.93 - 11.14X F=66.81 (1%)

FIGURE 6B

Fragments / 100 cells

Y = -15.00 + 75.77X F=725 (1%)

Normal division figures x

Y = 59.89 - 10.88X F=75.40 (1%)
FIGURE 7. Chromosome fragmentation, pollen abortion, and pollen germination following acute gamma irradiation of microsporogenesis. (A) control, metaphase I. (B) chromosome fragmentation at AI, 1kr. (C) fragments and bridging at AI, 2kr. (D)-(E) fragmentation at AI, 3kr, and 4kr. (F) chromosome bridging at AII. (G) control pollen. (H) pollen irradiated during PPI, 2kr. (I) pollen irradiated at PPI, 4kr. The germination of control pollen (K) is shown along with the germination of pollen irradiated at 2kr (L) and 4kr (M).
FIGURE 8. (A) A comparison of chromosome bridges resulting from a 16-hour irradiation with those induced in a 100-hour irradiation. The 16-hour series was irradiated at PI, the 100-hour series was irradiated from the PMC stage to AI; both were scored at AI. (B) The relationship between pollen abortion and chromosomal damage following a 16-hour irradiation during PPI of the meiotic division.
16 hour exposure
$Y = 2.34 - 0.81X_1 + 2.74X_2 \quad F = 55.25 (1\%)$

100 hour exposure
$Y = -0.85 + 7.17X \quad F = 685 (1\%)$

FIGURE 8A

% arcsin

Chromosome aberrations
$Y = 25.01 + 11.16X \quad F = 68.90 (1\%)$

Pollen abortion
$Y = 17.41 + 10.68X \quad F = 26.25 (1\%)$

FIGURE 8B
FIGURE 9. Pollen abortion following irradiation of different bud stages in *Quercus alba* and *Quercus coccinea*. The dotted line extrapolates from the sub-lethal to the lethal level for each bud stage irradiated.
FIGURE 9

\[ Y = 17.13 + 10.28x \]
\[ F = 308 (1\%) \]
\[ Y = 9.97 + 11.21x \]
\[ F = 617 (1\%) \]

Legend:
- \( \times \) Quercus alba PMC
- \( \triangle \) Quercus alba PI
- \( \triangle \) Quercus alba AII
- \( \circ \) Quercus coccinea PI
- \( \bullet \) Quercus coccinea AII
FIGURE 10. Germination and tube lengths of pollen from male buds irradiated at PI. Germination values are shown following an arcsin conversion of percentages.
Figure 10

Germination and Tube length

TOTAL EXPOSURE (kr/16 hours)