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CHRONIC AND ACUTE RADIATION OF MICROSPOROGENESIS

AND MATURE POLLEN IN QUERCUS

MASTER

by

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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 desireable 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 persistant 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 <u>Quercus</u>
were investigated under conditions of whole tree irradiation
The species utilized were <u>Quercus alba</u> Linn., <u>Quercus</u>
<u>ilicifolia</u> Wang., and <u>Quercus coccinea</u> Muench. The first of
these species is from the <u>Lepidobalanus</u> subgenus while the
latter two are assigned to the <u>Erythrobalanus</u> 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 15r/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 Co^{60} 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 $4^{\circ}C$; 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 $30^{\circ}C$; 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 8000r, 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 1000r/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 H_2O solution containing

20 ppm boron and was germinated at a temperature of 30^oC.

Tube lengths were measured on 20 germinating grains from

each treatment level after 24 hours.

<div align="center">CHRONIC IRRADIATION RESULTS</div>

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 15r/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). There-fore, 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 consider-
ably 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 consistant 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 Greenhouse are presented in a manner similar to Figure 6A. The number of chromosome fragments/100 cells ranged from 40 at o.5kr to 380 at an exposure of 5kr.

The intercept values (a) for both the acute and semiacute 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 consistantly 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 300kr. 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 100kr. At 200kr 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 300kr
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 200kr. At
200kr the tube growth was slightly suppressed and at 300kr
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 micro
beam 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 300kr 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 <u>Nicotiana</u> 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 <u>in situ</u>
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 <u>et al.</u> (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

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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 unaborted 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 R_1 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 LD_{50} 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 desireable. 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

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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
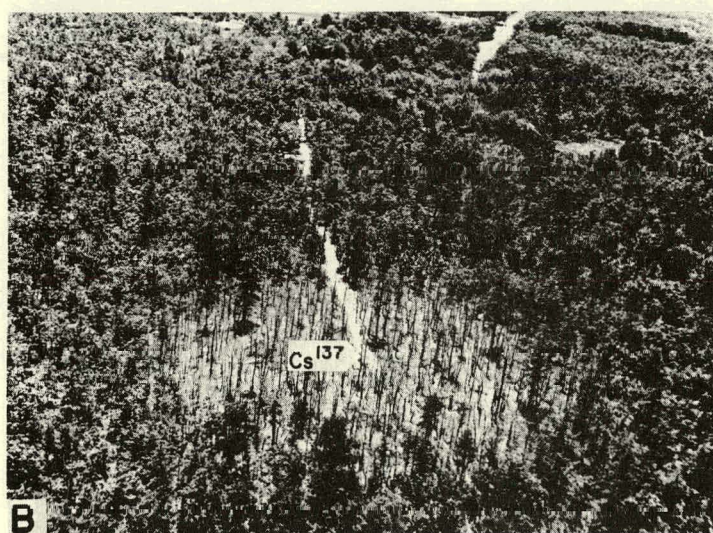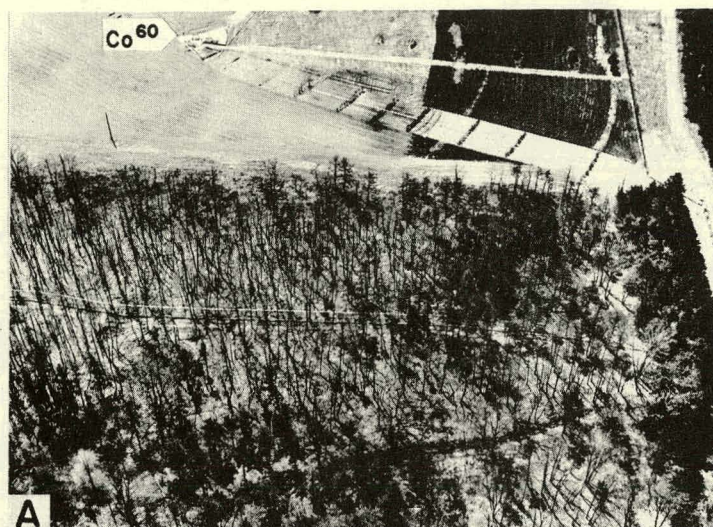season, that of the Gamma Forest (B) was taken
during early summer.

FIGURE I

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,
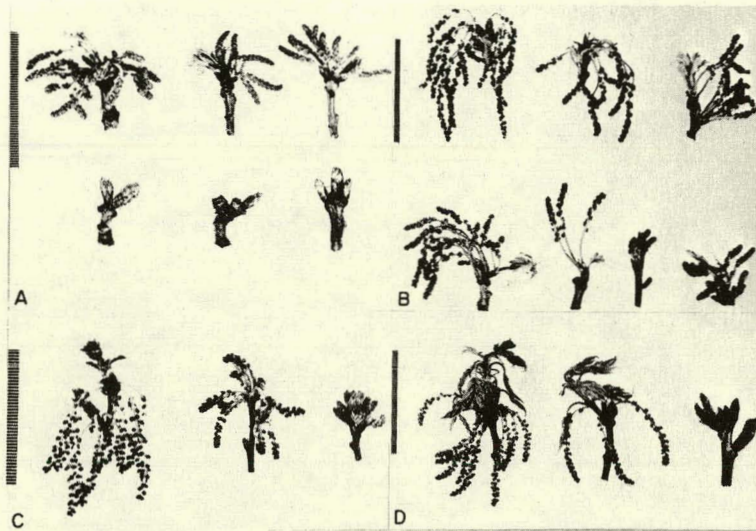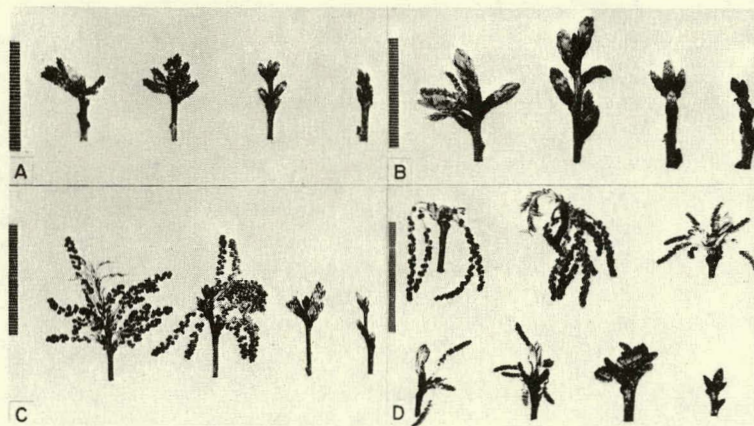84r.

FIGURE 2

FIGURE 3

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
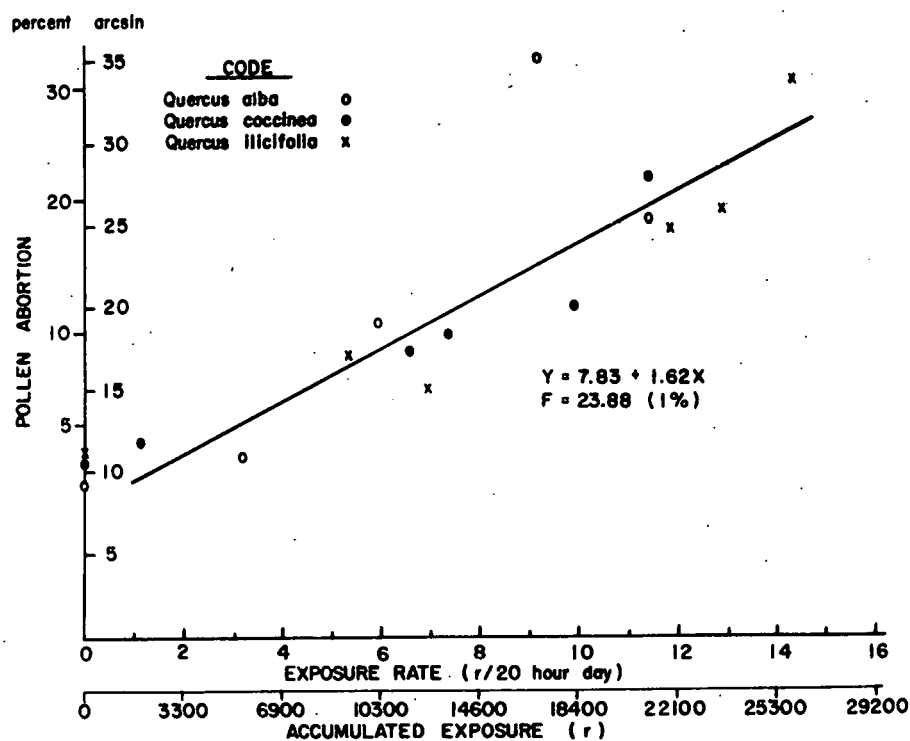   exposure of the mother trees is also indicated.

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, ploted over exposure
        rates per 20 hour day. The total accumulated
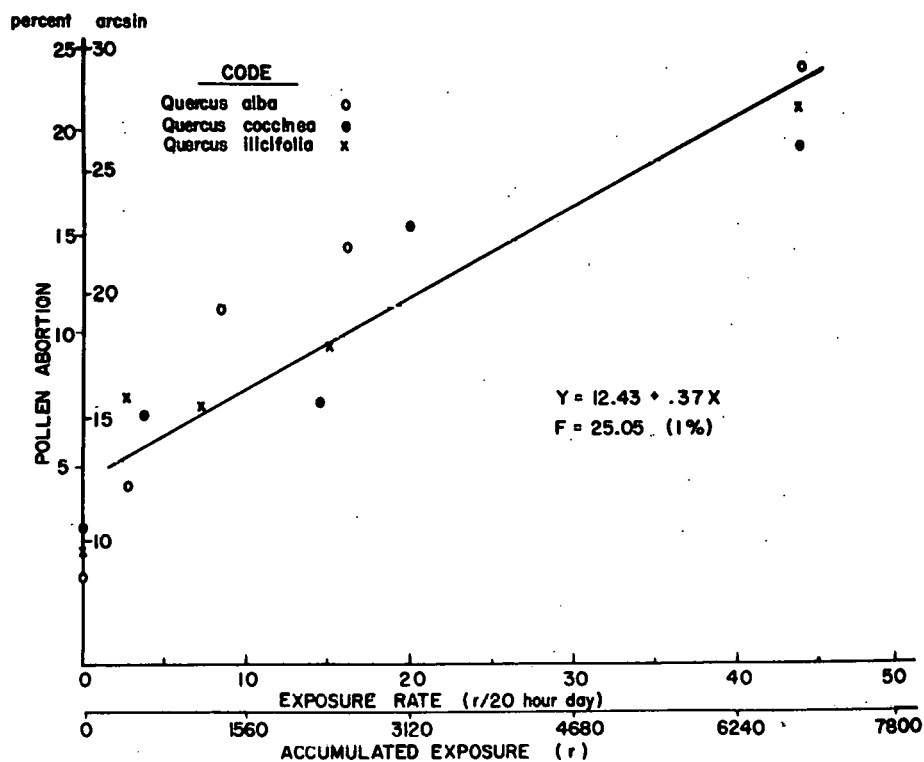   exposure of the mother trees is also indicated.

percent arcsin

CODE
Quercus alba   o
Quercus coccinea ●
Quercus ilicifolia x

POLLEN ABORTION

$Y = 7.83 + 1.62X$
$F = 23.88$ (1%)

EXPOSURE RATE (r/20 hour day)

ACCUMULATED EXPOSURE (r)

FIGURE 4

percent arcsin

CODE
Quercus alba   o
Quercus coccinea ●
Quercus ilicifolia x

POLLEN ABORTION

$Y = 12.43 + .37X$
$F = 25.05$ (1%)

EXPOSURE RATE (r/20 hour day)

ACCUMULATED EXPOSURE (r)

FIGURE 5

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

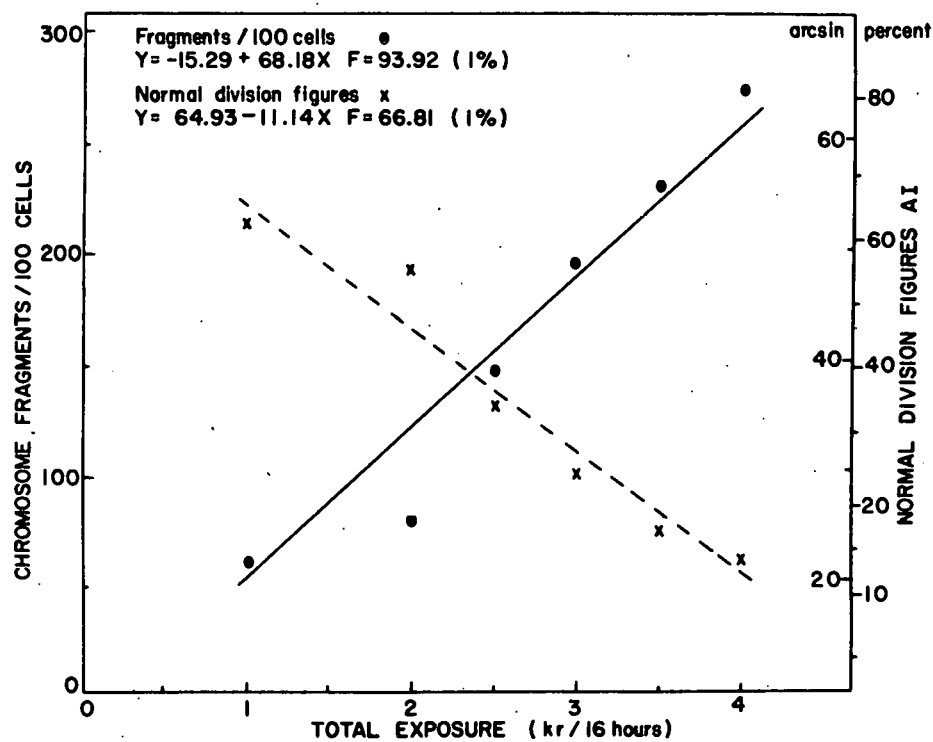at AI during meiosis. The total exposure
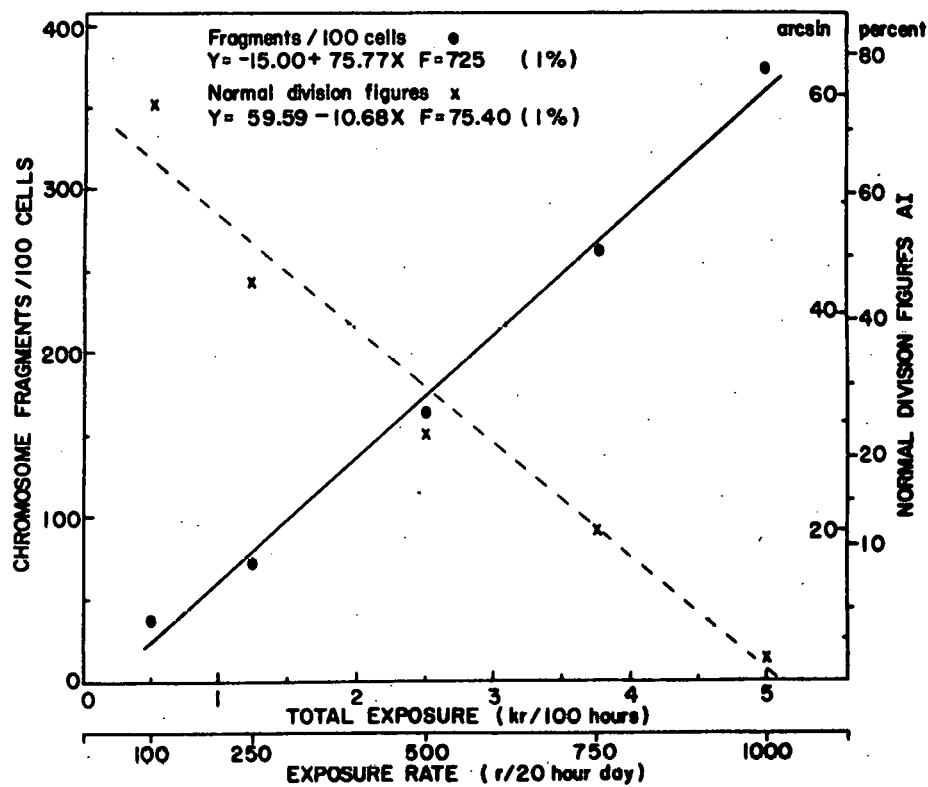
and exposure rate are given.

FIGURE 6A

FIGURE 6B

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) frag-
mentation 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
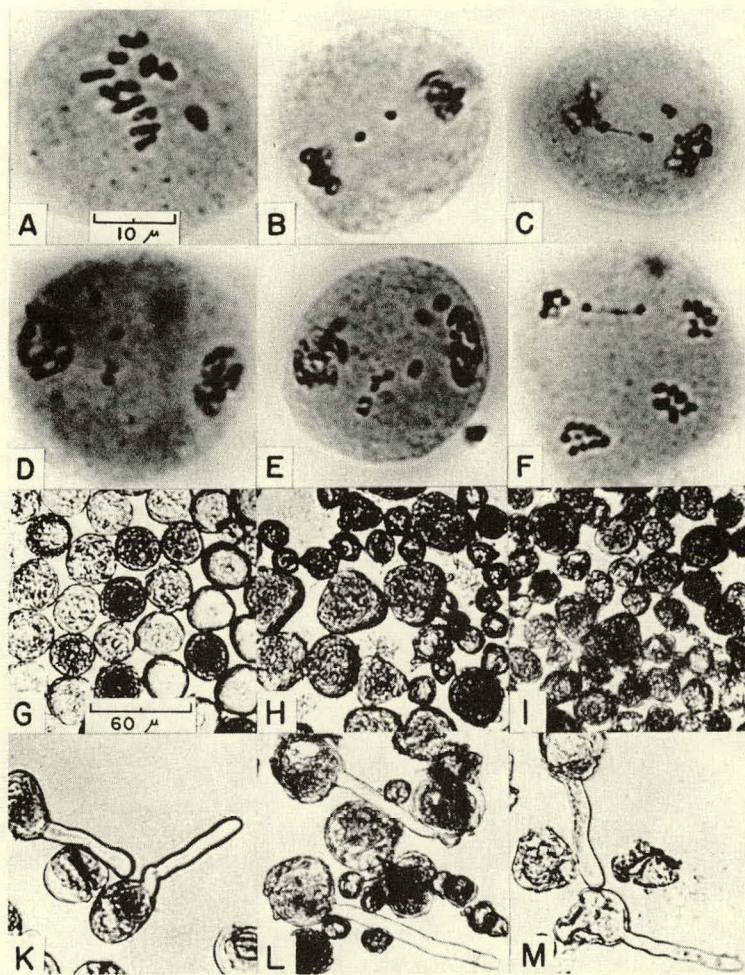irradiated at 2kr (L) and 4kr (M).

FIGURE 7

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
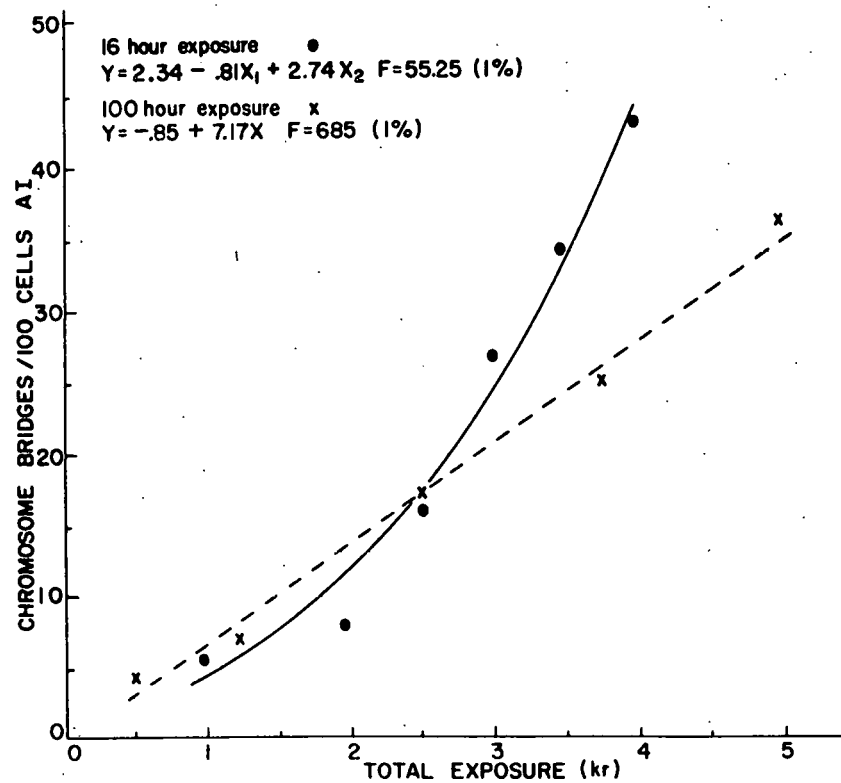
irradiation during PPI of the meiotic
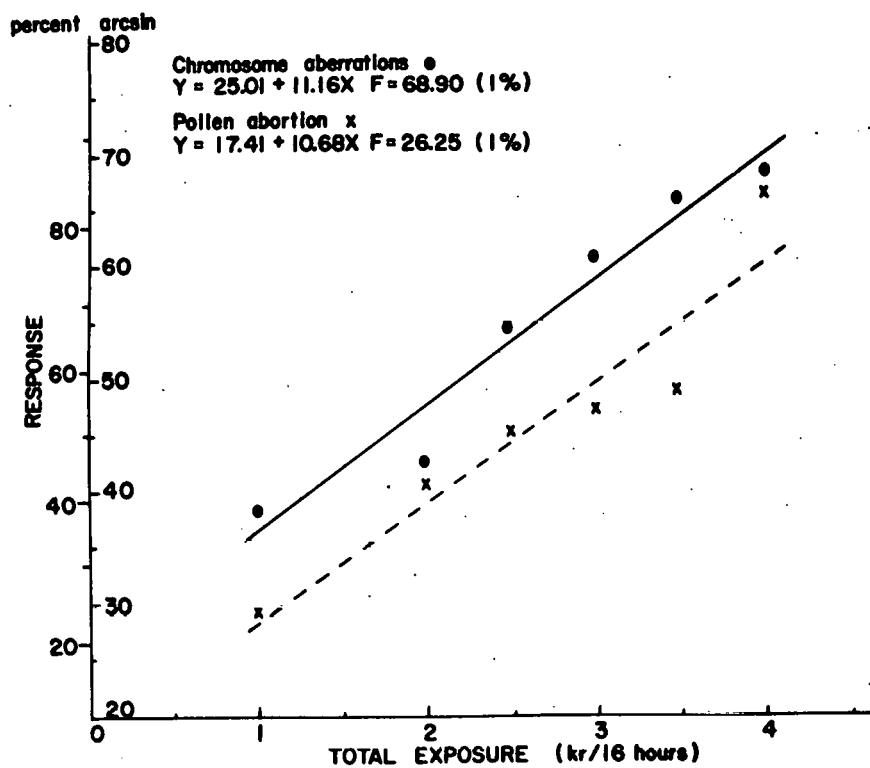
division.

FIGURE 8A

16 hour exposure ●
$Y = 2.34 - .81X_1 + 2.74X_2$ F=55.25 (1%)

100 hour exposure x
$Y = -.85 + 7.17X$ F=685 (1%)

CHROMOSOME BRIDGES /100 CELLS AI

TOTAL EXPOSURE (kr)

FIGURE 8B

percent arcsin

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

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

RESPONSE

TOTAL EXPOSURE (kr/16 hours)

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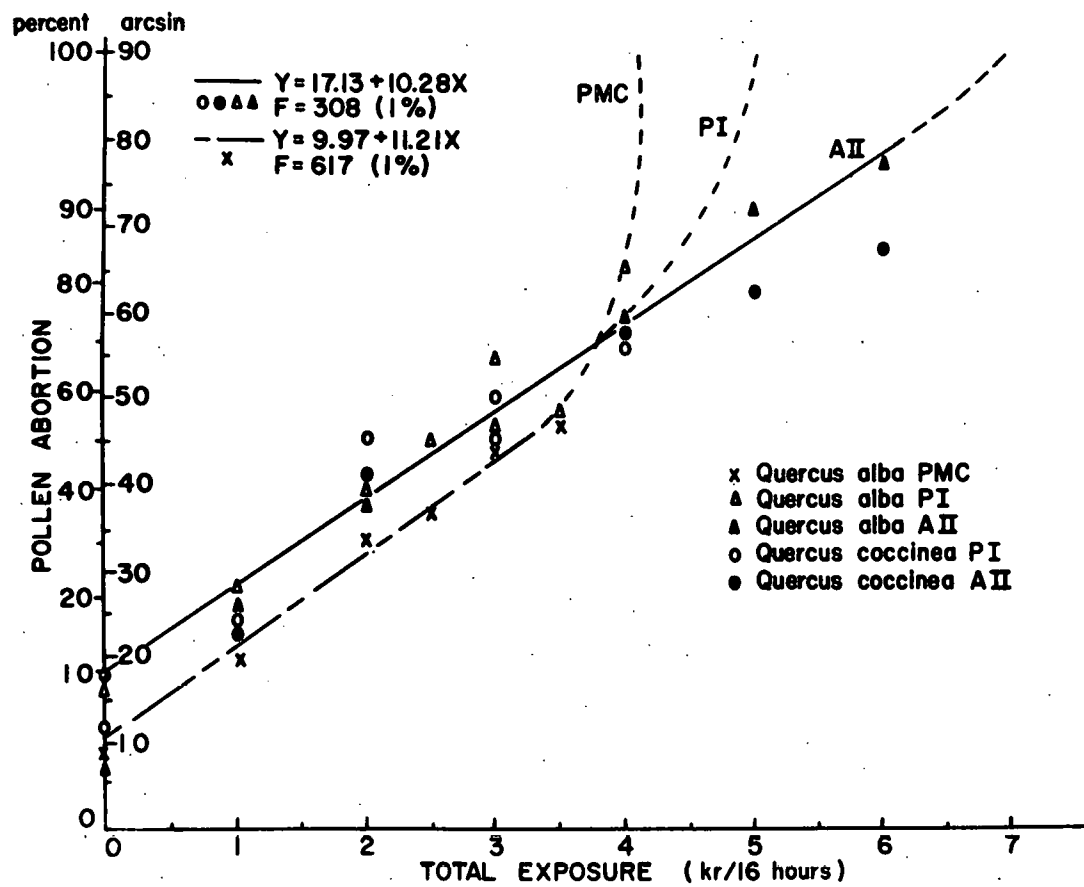the lethal level for each bud stage
irradiated.

FIGURE 9

FIGURE 10. Germination and tube lengths of pollen

from male buds irradiated at PI.

Germination values are shown following
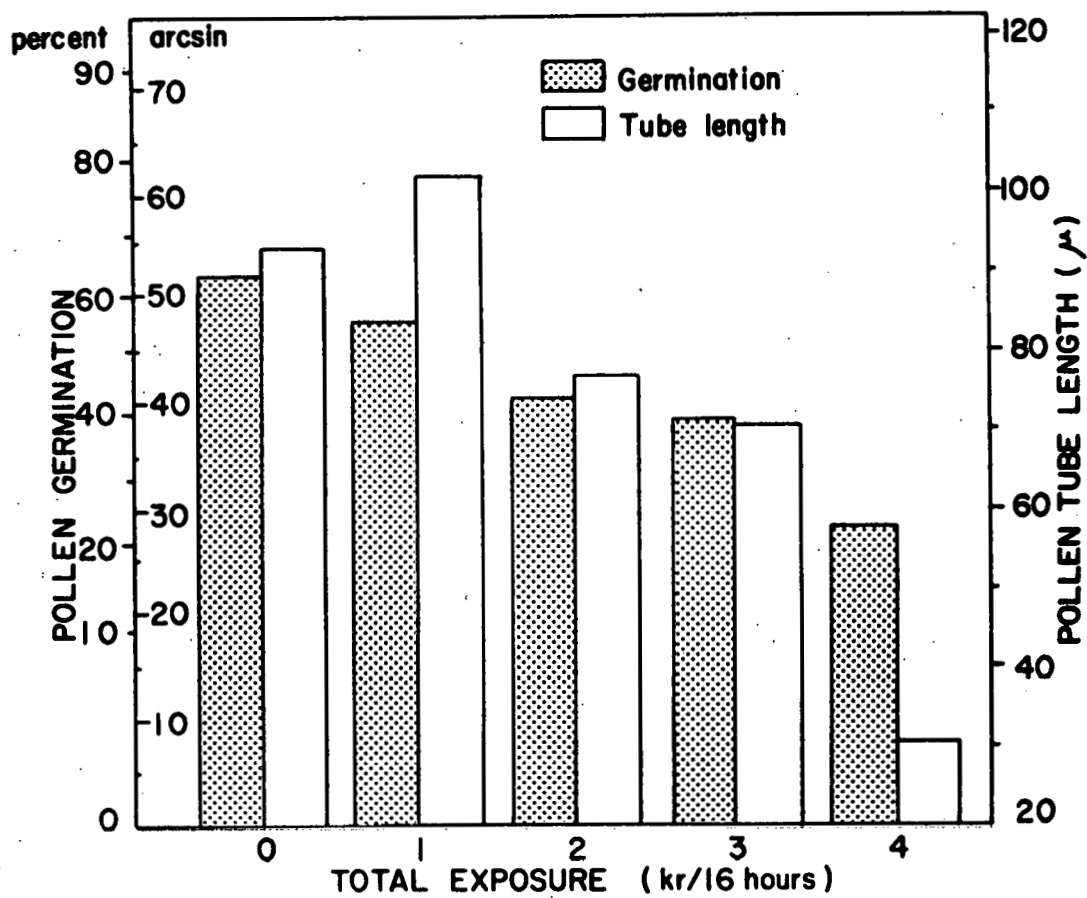
an arcsin conversion of percentages.

FIGURE 10