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ACUTE GAMMA IRRADIATION OF QUERCUS SEED - ITS EFFECT ON GERMINATION AND SEEDLING GROWTH

by

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DISCLAIMER

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INTRODUCTION

Dormant seeds are ideal material for studying the effects of ionizing radiation on plants because it is possible to modify the environment of the seed to a degree that would not be tolerated by other stages of the plant life cycle. By such means numerous investigations have shown radiosensitivity to be influenced by variation in genetic and physiological conditions, as well as by differences in the type and exposure rate of ionizing radiation utilized.

The international symposium concerning the effects of ionizing radiations on seeds held at Karlsruhe, in 1960, attested to recent advances and interest in this area of radiobotany. The proceedings of the meeting (International Atomic Energy Agency, 1961) contains current discussion on various aspects of seed irradiation. In addition to specific reports of experimental work, this publication includes a comprehensive review (Sparrow _et al_., 1961) of factors affecting the responses of plants to chronic and acute radiation exposure.

The publication of papers presented at a symposium on mutations and plant breeding (National Academy of Science, 1961) indicated the scope of current work in the field of induced mutations. In this symposium, Gaul (1961) reviewed the use of induced mutations in seed propagated species; while an article by Sparrow (1961) discussed the cytogenetic effects of ionizing radiation.

Information concerning the effects of ionizing radiation on forest trees has come principally from seed irradiation experiments, and the majority of these studies have dealt with defining radiosensitivity of conifer seed; only a few reports are for deciduous species. The data reported show considerable variation between studies dependant upon species, type of radiation, rate of exposure, and total exposure.

An exposure of 2000r x-radiation was lethal to *Pinus palustris* and *Pinus taeda* (Snyder *et al.*, 1961). Gustafsson and Simak (1958) observed a reduction in germination by 92 percent in *Picea* after exposing dry seed to 1200r; seed of high moisture content showed no germination reduction at this dosage. May and Posey (1958) reported 90 percent germination of *Pinus elliottii* seed after irradiation of 500r from a Co^{60} source, and 10 percent following 25000r. Less than 30 percent germination of x-radiated *Pinus sylvestris* seed was reported at a level of 3600r (Suszka *et al.*, 1960). Vidakovic (1960) reported stimulation of germination by gamma radiation at levels as high as 4000r for *Picea abies*, 3500 for *Pinus nigra*, and 2500 for *Pinus sylvestris*. In addition to references mentioned above, recent papers have illustrated other factors leading to variation of radiation effects on forest tree seed.

Included among these are the effect of water content (Ohaba, 1961), provenance differences within the same species (Ohaba and Simak, 1961), and seed weight (Simak et al., 1961).

Heaslip (1959) reported a lethal level of 10000r for seed of Quercus alba irradiated with Co^{60} gamma rays. At this same radiation level she reported 1.2 percent germination of Quercus prinus and 16.8 percent for Quercus velutina. Report of Populus seed irradiation (Jovanovic and Tuconic, 1960) has shown it to be relatively resistant to radiation. These authors found little effect on germination with gamma radiation exposures up to 50000r; above 50000r no surviving seedlings were obtained. Scholz (1957) has shown that the term LD_{50} may be misleading in seed irradiation studies unless survival values are included. In his study of mutation induction in birch, survival values of seedlings from irradiated seed still showed radiation effects after several years.

The wide variation obtained by different investigators indicates a need for exact definition of materials and methods used. In addition it would seem advisable to qualify seed irradiation reports by observation of the seedlings following the first flush of growth.

Experiments reported in this study were conducted to evaluate radiosensitivity of Quercus seed to acute gamma radiation. The data reported includes information of some cytological and morphological effects in seedling growth following seed irradiation. Investigation of varying seed moisture contents and physiological state at the time of treatment is included as well as preliminary evidence of induced mutations.

MATERIAL AND METHODS

General

The seed of two species representing two sub-genera in the genus *Quercus* was selected for irradiation. The *Lepidobalanus* subgenus was represented by *Quercus alba* Linn., while *Quercus rubra* Du Roi was selected for the *Erythrobalanus* group. *Quercus alba* seed was collected at the Brookhaven National Laboratory site, Upton, New York; while that of *Quercus rubra* was obtained from trees in the New Haven, Connecticut area. In both species treatment was made on mature seed of the current years crop. Seed of *Quercus alba* germinates readily at maturity, and may begin radicle emergence while still on the mother tree or shortly after falling. For this reason it was necessary to reduce the moisture content slightly prior to storage. Ungerminated seed was selected for radiation treatment after approximately 30 days of storage at $4^{o}C$.

Quercus rubra seed requires no vernalization prior to germination and does not normally begin emergence until placed in a warm, moist environment. To attain maximum germination the seed was allowed to after-ripen at $4^{o}C$ for 45 days before irradiation.

Experiment I

Cytological and Morphological Evaluation of Radiosensitivity

Seed from each of the two species were selected for general uniformity of size, weighed to evaluate the possible correlation between seed size and growth, and then randomly divided into two groups. The moisture content at the time of irradiation was 45 percent for Quercus alba and 27 percent for Quercus rubra.

Group I was placed in 11 packets of 45 seed each for treatment at the following levels: control, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 10kr. Radiation was delivered over a 16-hour-period from a Co^{60} source. Within 6 hours after radiation the acorns were sown in a 3:1 mixture of sand and peat moss and allowed to germinate under controlled greenhouse conditions (25°C days, 19°C nights) with a day length of 18 hours. Statistical design was randomized by treatment rows within wooden flats. Germination was scored at epicotyl appearance above the soil surface and height measurements were made at 5-day-intervals; initial shoot elongation was completed some 20 days after germination. The appearance and point of origin of sprout growth was also recorded.

Group II seed was prepared as was seed of Group I and exposed to radiation at the following levels: control, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 32kr. Since this group was intended for observation of root growth and histological examination of root and shoot development, the acorns were placed in flats filled with sphagnum moss. The flats were then placed under environmental conditions similar to Group I seed. At the time of germination, root and shoot tips were cut from 5 individuals at each level and fixed in F.A.A. The material was embedded in tissue-mat and sectioned at 8 microns; sections were stained with safranin and fast green. The remaining seeds were evaluated for shoot emergence, and for root growth at 5 and 10 days following inception.

Experiment II

The Effects of Varying Moisture Content and Physiological State on Seed Sensitivity

Three pre-irradiation conditions were established with seed of *Quercus rubra*. To evaluate varying moisture contents and different physiological states at the time of radiation, the following conditions were established: (1) seed stored at 4°C with a moisture content of 22 percent, (2) seed placed in a saturated atmosphere at 4°C for 10 days with a final moisture content of 44 percent, and (3) seed incubated at 29°C for 10 days with a moisture content of 47 percent. The latter treatment was designed to provide material in an active metabolic state previous to actual cell division. Seed was placed in plastic bags to maintain moisture conditions during the radiation exposure; 25 seeds were selected for each exposure level. A 16-hour gamma irradiation treatment from a Co^{60} source provided the following total exposures: control, 1, 2, 4, 6, 8, 10, 12, 16, 32kr. The seed was sown in sphagnum moss flats approximately 6 hours after irradiation, and germination proceeded under controlled greenhouse conditions. Root elongation was recorded for 5 and 10-day periods, and shoot emergence was recorded for each level.

EXPERIMENTAL RESULTS

Experiment I

The time required for germination was similar for the two species; in both the root began growth first, followed by initiation of shoot growth in 5 to 10 days. Early growth of the root in length was rapid; in the sphagnum moss flats the controls elongated at a rate of approximately 1.5 cm/24 hours during an initial 10-day period. Development of the shoot was also quite rapid, and the bulk of height growth was completed after 20 days. The beginning of growth in the apical meristem is contingent upon endogenous factors and not upon ultimate root development. In support of this it was observed that severing the root at emergence did not prevent growth of the shoot; lateral roots were formed at the point of cutting and quickly regenerated a ramified system of branch-roots. *Quercus rubra* grew to a larger size more quickly than *Quercus alba*. The correlation between seed size and seedling height was not significant either for the control or irradiated groups, but this may be due to previous selection for uniform seed size.

The time required for germination varied between levels but was not significantly related to radiation exposure. In some experiments the irradiated seed germinated more quickly than the control; however, this trend was not consistant enough to be interpreted as a definite stimulation effect. Total germination also varied between the two species both in the control and irradiated groups. In Quercus alba germination of the control group was 95 percent and a linear decrease was obtained with increasing radiation levels. (see Figure 1A). An LD_{50} was obtained at approximately 6kr and total germination at 10kr was lowered to 25 percent. In Quercus rubra germination of the control group was 70 percent; however, the regression of germination on radiation level was not significant at the .05 level (Figure 1B). There was a gradual decrease in total germination and a significant difference was obtained in comparing the 10kr level (20 percent germination) with the control.

The wider range of exposures in Group II of Experiment I included the lethal point for both species. No shoot emergence was observed at 16kr for either species, although a limited amount of root elongation took place at the 16 and 32kr levels. Germination at 12kr was sporadic and comprised less than 10 percent of the seed irradiated. Thus the root growth immediately following germination was less inhibited at the higher radiation levels than was shoot growth.

Survival values for both species were obtained at the end of the first growing season. The calculated regressions for survival indicate significance at the .01 level for *Quercus alba* (Figure 1A) and non-significance for *Quercus rubra* (Figure 1B) when plotted over radiation exposure. The elevation of the regression lines for survival were not significantly different from those for germination. Thus the values obtained are simple reflections of germination percentages.

The total height growth obtained at different treatment levels for both species in Group I is shown in Figure 2A and 2B. Two general patterns of height growth were observed: (1) shoot growth, resulting from continuous growth of the original apical meristem and, (2) sprout growth, from either axillary or adventitious primordia. The term adventitious sprouting is herein used in the broad sense; any damage to the original apical meristem resulting in reorganization and subsequent growth from such areas was considered adventitious sprouting.

Curvilinear regressions of the second order polynomial ($y = a + bx + cx^2$) were calculated for sprout and shoot growth separately. The relative frequency of individuals represented by each point on the graph decreases with increasing exposure for shoot growth, and increases at the higher levels for sprout growth. The number of individuals that sprouted in response to radiation damage varied from 6 percent at 1kr to 75 percent at 6kr for *Quercus alba*, and 5 percent to 93 percent for Quercus rubra, at similar levels. Because of the wide variation between total heights at differing levels and unequal survival at the different levels, the overall regression was not significant (.05 level) for either species or for type of growth. Comparison between species is complexed by inherent differences of growth pattern, and within species by physiological factors concerned with type of shoot growth. Therefore, height growth did not give a reliable indication of radiation damage. Among individuals where radiation damage had induced sprouting, the height of shoot growth prior to sprout formation decreased with increasing levels. Examples of the types of sprouting observed are shown in Figure 3A-C and quantified in Figure 4. The similar reaction by the two species in response to radiation damage is shown by the parallel regression lines in Figure 4. The significant difference (1 percent level) in elevation between the regressions reflects variation in shoot growth inherent to each species.

The physiognomy of fully developed seedlings in the irradiated groups was much more variable than the controls. In Figure 3D the range of variation in the irradiated group is shown in contrast to the control seedlings (Figure 3E). The types of aberration observed were: changes in leaf size, shape, and texture; contorted stem growth; and reduction in overall growth. Dwarfing was common at the higher levels of radiation and there were also changes in chlorophyll content indicated by abnormally dark or pale leaves. Only one individual from the irradiated population was outstanding in total height growth compared to the control group, although many were as large or slightly larger than the controls. This seedling from a seed irradiated at 5kr underwent 3 successive growth flushes during the first growing season prior to hardening off in the fall. Total height was 34 cm compared to a maximum of 20 cm for the tallest in the control group. Secondary flushing is common in oaks and several of the control and irradiated seedlings produced multiple flushes; however, none were as vigorous as the single seedling mentioned. At the end of one growing season some aberrant types appeared to be mutant forms, but their ultimate evaluation properly awaits subsequent growing seasons. All of the seedlings from the irradiated seed have been outplanted in a test nursery to allow future evaluation.

Histological examination of apical growth was made from seedlings in Group II, Experiment I. Microtome sections from material collected at germination and at intervals through the elongation period indicated that growth at higher levels (7kr and above) originated from sprouting directly at the root collar. At these levels the embryonic apical meristem was so damaged by radiation that normal growth did not take place. Instead, reorganization of apical primordia occurred at several points within the multi-celled meristem, resulting in the growth of one or more adventitious sprouts.

Examples of the external appearance of a control and two irradiated individuals are shown in Figure 5A-C and longitudinal sections are illustrated in 5D-F. At lower radiation levels the shoot growth was less effected and the sprouting that occurred came from axillary meristems. Subsequent internal organization and establishment of axillary primordia in the elongating sprout proceeded in the usual manner.

Root growth of the two species was analysed in Group II, Experiment I. A linear decrease in elongation rate was directly associated with increasing radiation exposures. This response is shown for Quercus alba in Figure 6A and for Quercus rubra in Figure 6B. In the two graphs root length is plotted as the dependant variable over the logarithm (base 10) of radiation exposure. Root length after 10 days was similar for both species, despite an earlier difference between species at 5 days. This descrepency and the difference in intercept values comparing growth periods between species reflects different inherent growth rates and is not a radiation effect per se.

Examination of histological sections of developing root tips indicated that the limited growth observed at 32kr was due almost entirely to cell elongation. At this radiation level an initial sporadic wave of cell division occurred during radicle emergence but no further mitotic activity was observed. In the remaining levels there was a positive correlation between mitotic activity and root length. However, due to an apparent difference in endogenous times of peak cell division among individuals, root length was selected as the best estimator of total mitotic activity. In Figure 5G-I longitudinal section of root tips from a control and two irradiated levels (8kr and 16kr) are shown. These photo-micrographs illustrate a decrease in meristematic area caused by radiation damage of embryonic primordia. Maturation of individual cells occurred closer to the apical meristematic regions of irradiated roots than in the controls. However, the slower cell divisions of irradiated individuals was the physical factor for this phenomenon and not accelerated cell development. Cross section of mature root segments showed a normal pattern of vascular differentiation except at 16 and 32kr; because there was no shoot emergence and only slight root growth at these levels, it may be assumed that they constituted lethal exposures.

Experiment II

Experiment II was conducted to evaluate the effect of varying moisture content and physiological factors upon seed radiosensitivity. Root length during the first 10-day-period following germination had been established as a reliable indicator of radiation damage and this method was utilized to evaluate the three experimental conditions. The results of Experiment II are plotted in Figure 7. Here the length of root growth is plotted over the logarithm (base 10) of exposure level in kr. In a preliminary analysis, separate regressions were calculated and an analysis of variance for difference between the three pre-treatments was non-significant at the 5 percent level. Therefore, single regression lines were calculated for root growth during each period. Shoot emergence from the three groups followed the pattern previously discussed in Experiment I. Only limited shoot emergence (less than 10 percent) occurred at 12kr and none at 16 and 32kr. Again the root development at 16 and 32kr was mainly by cell maturation and elongation with little mitotic activity taking place. An analysis of variance for regression lines obtained in this experiment, compared with those from Experiment I yielded no significant difference (.01 level) for slope (b) or intercept (a). Thus the assay method for radiation damage utilizing the initial 10-day root growth was consistent over replicated trials.

DISCUSSION

The lethal exposures were similar for both species and were expected from *a priori* consideration of their similar nuclear volumes (see Sparrow *et al*., 1961). However, the different germination results within treatment levels, and between species, is less easy to explain. The results are complexed by physiological and genetical interaction in response to radiation treatment, and direct separation of the two is difficult.

The reports by Gray and Scholes (1951) and by Read (1959) have shown the meristematic areas to be the site of plant radiosensitivity. At the cellular level, the greater importance of nuclear to cytoplasmic considerations has recently received review (Sparrow and Evans, 1961; Evans and Sparrow, 1961). Within the nuclear hypothesis the sensitivity of DNA (see Lajtha, 1960; Setlow, 1961). RNA (see review by Kelly, 1961) nuclear phosphorylation (Creasey and Stocken, 1959), and nuclear volume (Sparrow and Miksche, 1961) are particularly important aspects. Sparrow and Miksche (1961) showed that the radiosensitivity of a large number of plant species is closely correlated with nuclear volume and DNA content. Although nuclear radiosensitivity is a major factor, the sensitivity of cytoplasmic enzyme reactions need also to be considered. Gordon (1957) has pointed out the radiosensitivity of indoleacetaldehyde conversion to indoleacetic acid, In general protein synthesis has been reported to be less affected by radiation (see Kelly, 1961) than the more sensitive nuclear components.

The variation of germination between species may reflect interaction of radiation with any of the above systems. The data from individuals which grew by sprouting also suggests such modification. The amount of shoot growth prior to sprout origin showed a linear decrease with an increase in radiation. This effect may be an expression of altered physiology which is repaired during the cytological sequence leading to sprout growth. Although obviously a complex relationship, the variation in germination values and at least a major component of the sprouting reaction would seem to be a physiologically conditioned response.

Because the root and shoot growth values are similar in both species it would appear that genetic reaction of the two species to radiation was also the same. Shoot growth was complexed by sprouting in response to radiation damage and thus was not a good indicator of radiation effect. Pinus rigida seedlings exposed to gamma rays showed a similar response, and sprouting was a common phenomenon in the plants damaged by radiation (Mergen, 1963). Root growth showed a linear decrease with increasing radiation exposure and provided an approximation of genetic response. Growth of the root may be less sensitive to physiological environment or at least less demanding in the need for endogenous growth substance and general biological harmony than is epicotyl growth.

The interpretation of Experiment II concerning different moisture contents and physiological condition at the time of irradiation was also based on root elongation. The root length in this experiment showed little fluctuation in response to pretreatment; a fact which lends support to its use as a macro-measure of genetic damage. It is quite likely that similar results will be obtained in irradiation of other hardwood species that have an inherent sprouting ability. The use of early root growth may be used as one measure of radiation response in such species.

The uniform survival values obtained were dependant upon the recovery of apical meristems from radiation damage by sprout growth. Such somatic selection among genetically damaged cell populations has been called diplotonic selection by Gaul (1959, 1961). Axillary sprouting arising from primordia laid down in the normal growth pattern was the most common form of recovery at lower levels of radiation. Results at the higher levels (7kr) indicated an early competition among apical meristematic cells with subsequent organization of adventitious sprouting, Actually there was no distinct threshold exposure at which one form of sprouting was exclusive to the other; diplotonic selection was no doubt a factor at all levels of treatment, with increasing magnitude as the amount and severity of cell damage increased.

Competition among cells indicates the chimeral nature of the embryo following irradiation. Whether the induced genetic variability within seedlings will persist with further growth depends on the number of cells involved in primordial activity. In the case of multiple adventitious sprouting it is quite likely that they would be genetically dissimilar. Moh (1961) has suggested that the apical growth of a coffee plant results from proliferation of a single embryonic cell. If the situation is similar in Quercus then any particular sprout or shoot would be genetically uniform. The aberrant forms observed during the first growing season included many plants with uniform changes in all the leaves; although, in some a portion of the leaves were aberrant while others on the same plant were normal. The latter suggests a chimeral origin which may subsequently segregate as growth progresses.

The range of radiation exposure from 1 to 10kr is recommended for use in induced mutation studies with oak seed. The moisture content normally found in oak seed is high, and variation above 25 percent did not change the radiosensitivity. In terms of the most consistant macro-estimator of genetic damage (root growth) increased metabolic activity prior to germination was also ineffective in altering sensitivity. The similar genetic reaction by both species investigated relates to their comparable nuclear and chromosomal complements; any differences observed in this report can be explained in light of physiological diversification. Results obtained may be applicable to a large number of woody angiosperms of similar nuclear makeup, although variance due to inherent physiological differences is anticipated.

SUMMARY

Dormant seed of *Quercus alba* and *Quercus rubra* were irradiated with gamma rays from Co^{60} to determine the effect on cytological and morphological expression in the resulting seedlings. Investigations included varying moisture content and physiological state of the seed prior to treatment, and preliminary evaluation of induced mutation. The following observations were made.

1. The lethal radiation exposure was the same for both species although there were differences in germination between species within the treatment range of 1 to 10kr. *Quercus alba* showed an LD_{50} of 6kr with a significant regression for radiation level. The regression of germination on radiation level was not significant for seed of *Quercus rubra*, but germination was suppressed at the higher levels. These species differences are thought to be due more to physiological than genetic factors.
2. Height growth in the two species was complexed by sprouting in reaction to radiation damage of the apical primordia. Seedling height at the end of one year was not a significant measure of radiation damage. The type of sprouting obtained is discussed in relation to diplotonic selection within the irradiated embryonic primordia.
3. Growth of the root at 10 days following inception was established as a macro-measure of genetic damage. Changes in moisture content (above 25 percent) or metabolic activity

prior to germination did not significantly change the response of root growth to radiation exposure. It is suggested that initial root growth is useful as a bioassay of radiation effect on the seed of woody angiosperms where sprouting is common.

4. The range of 1 to 10kr is recommended for use in oak seed irradiation experiments for the induction of mutations. Preliminary evidence at the end of the first growing season indicates that most aberrations observed are generally deleterious to total growth. However, many of the irradiated seedlings were as large as the controls and at least one irradiated seedling was outstanding in total growth.

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FIGURE 1. Relation of germination and survival to acute radiation exposure of dormant seed in *Quercus alba* (A) and *Quercus rubra* (B).

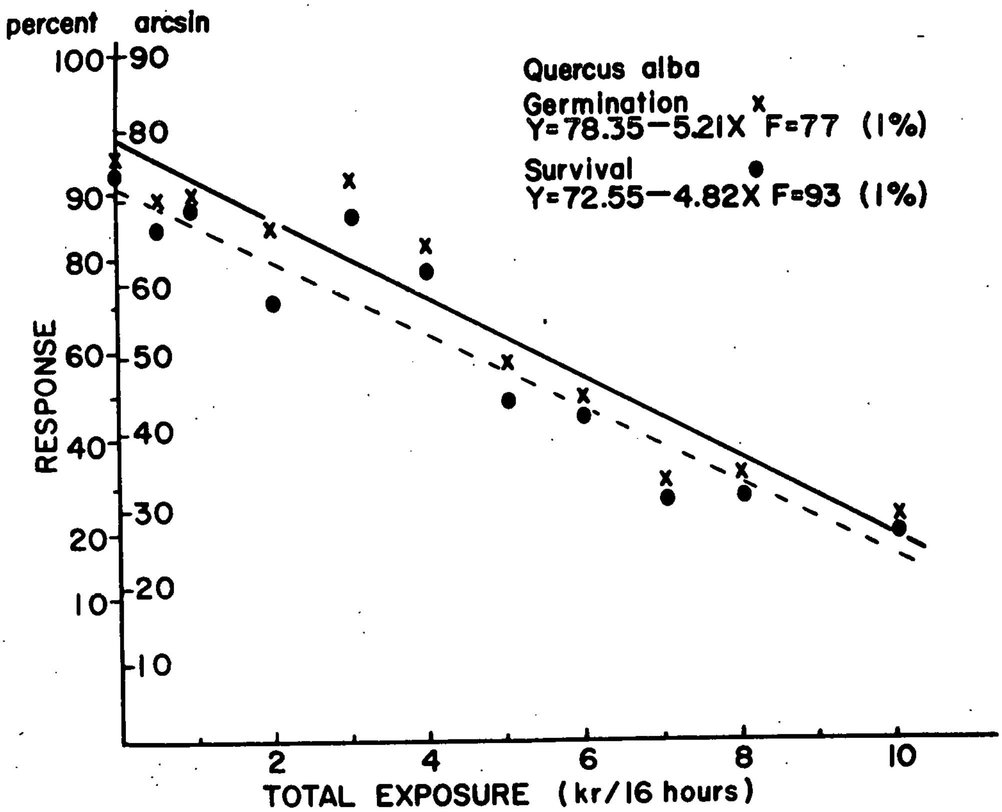


FIGURE 1A

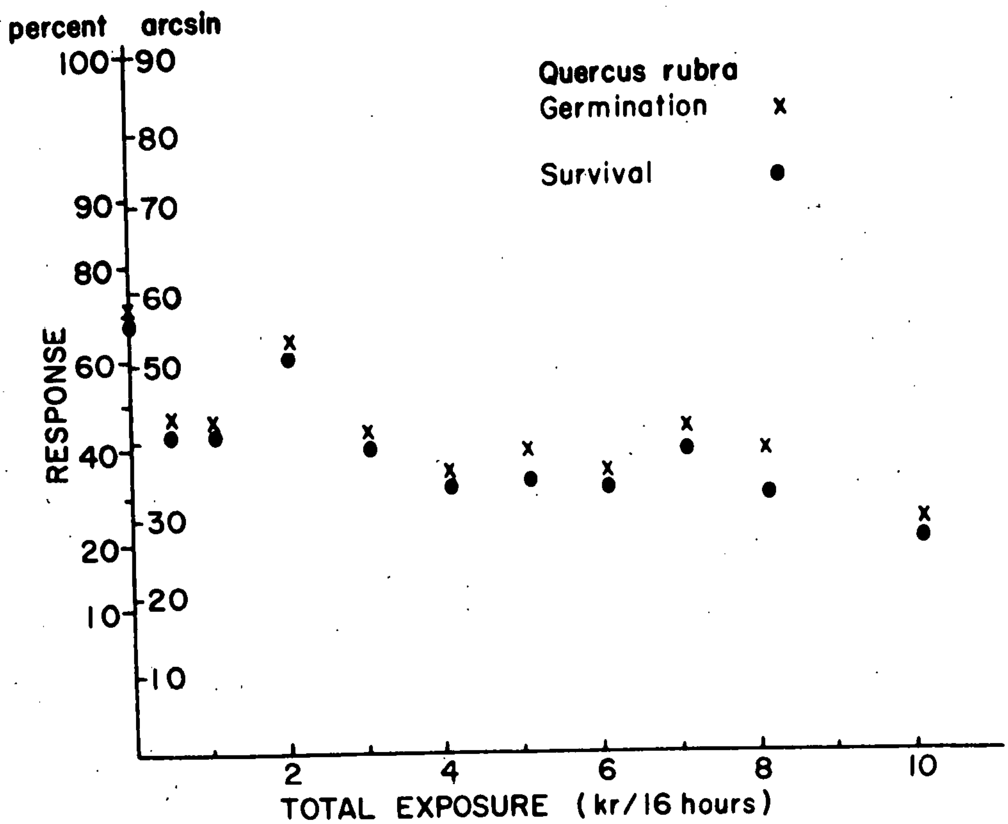


FIGURE 1B

FIGURE 2. Height growth of seedlings from irradiated seed, a distinction is made between shoot and sprout growth for *Quercus alba* (A) and *Quercus rubra* (B).

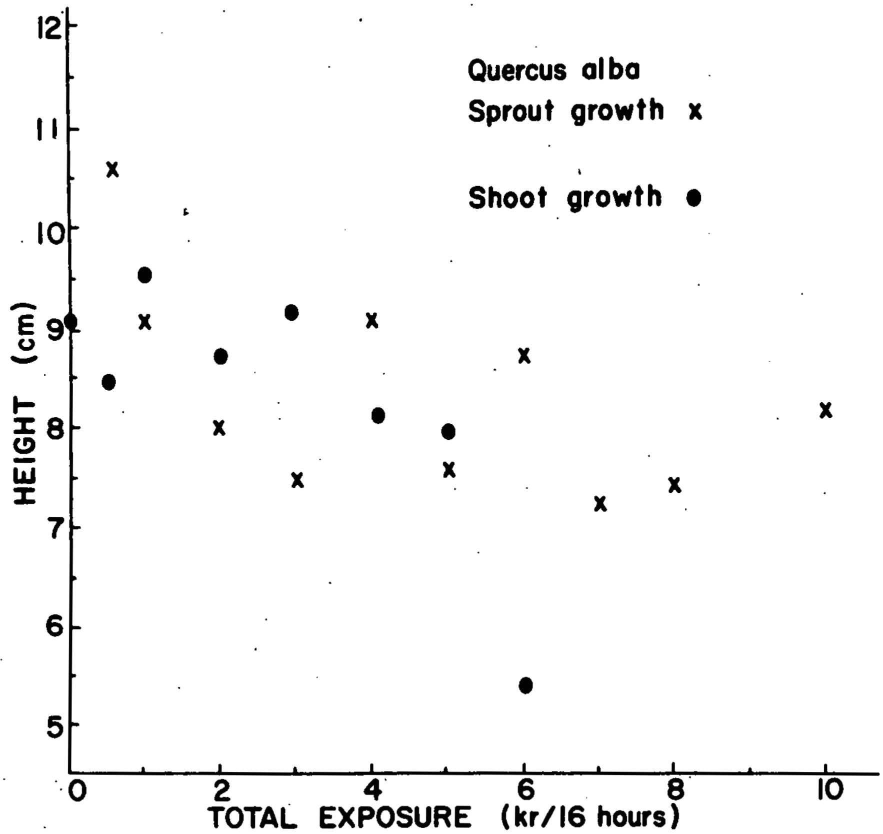


FIGURE 2A

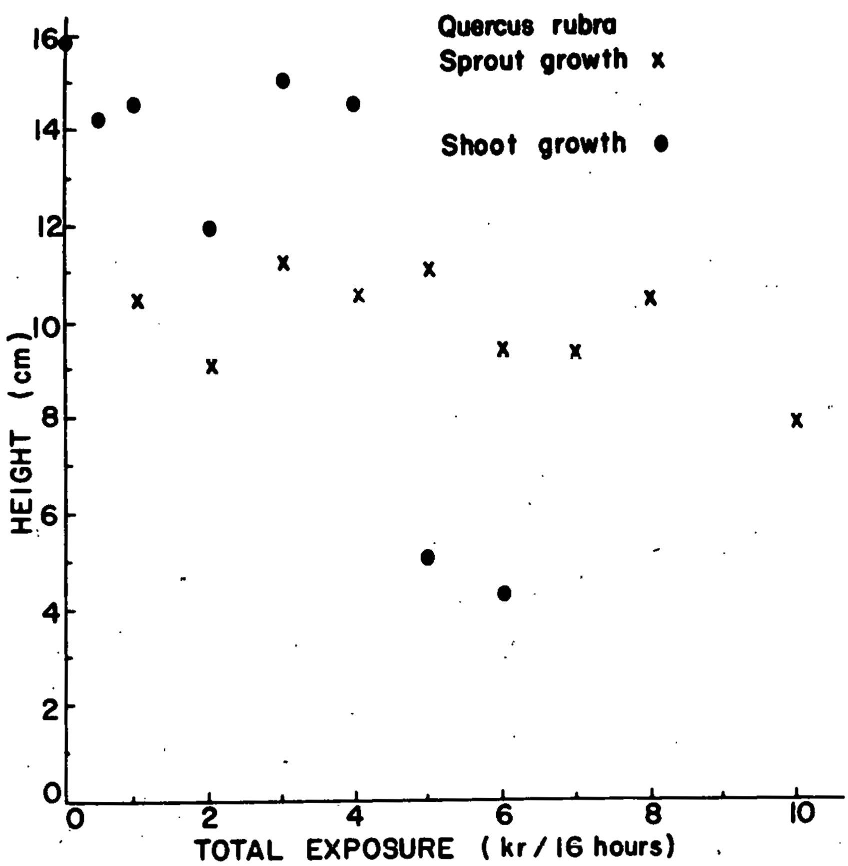


FIGURE 2B

FIGURE 3. Growth of seedlings from irradiated seed. (A to C) Sprouting at 1kr (A), 4kr (B), and 7kr (C). The range of variation in an irradiated group (D) is compared to the control (E). The seedlings are in 4 inch pots.

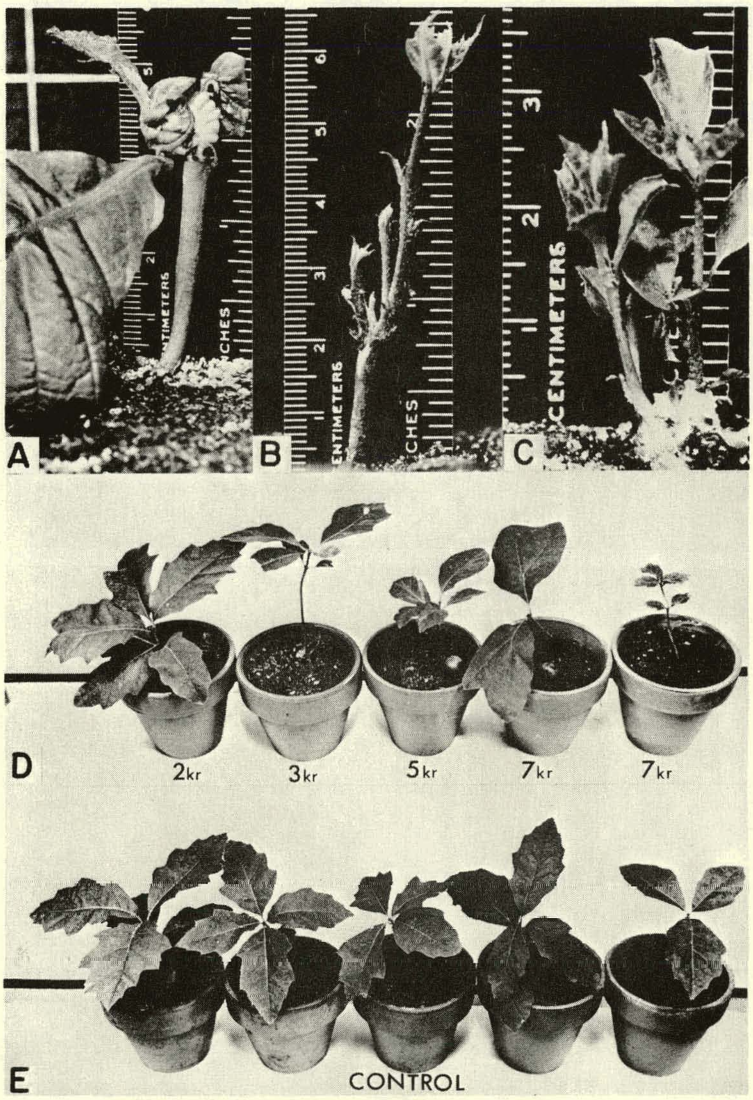


FIGURE 3

FIGURE 4. The mean height at which sprouting occurred due to radiation damage of embryonic primordia in the dormant seed.

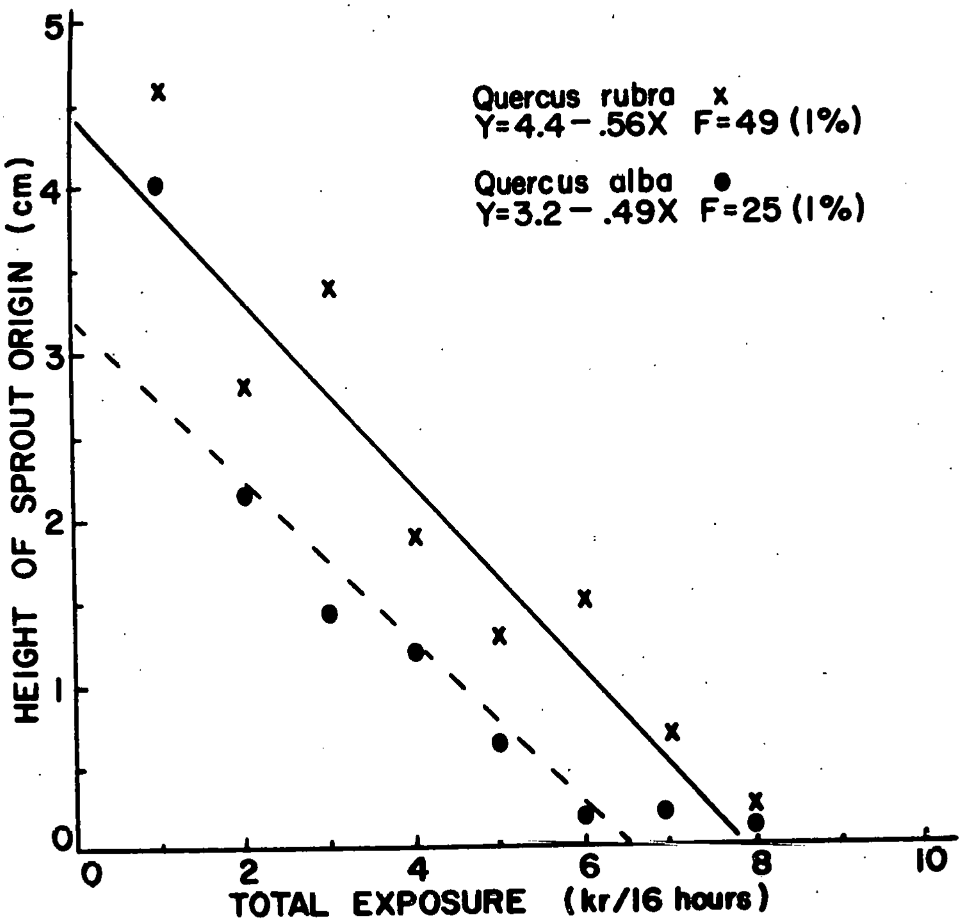

Quercus rubra x
Y=4.4−.56X F=49 (1%)
Quercus alba ●
Y=3.2−.49X F=25 (1%)
HEIGHT OF SPROUT ORIGIN (cm)
5
4
3
2
1
0
0
2
4
6
8
10
TOTAL EXPOSURE (kr/16 hours)

FIGURE 4

FIGURE 5. Morphology of early shoot and root growth from irradiated seed. Shoot segments excised at the root collar are shown for a control (A), 7kr (B), and 12kr (C). Longitudenal sections from the same exposure levels are shown directly below (D to F). Sections of roots excised at germination are indicated for control (G), 8kr (H), 16kr (I). The arrows indicate the region of mitotic activity.

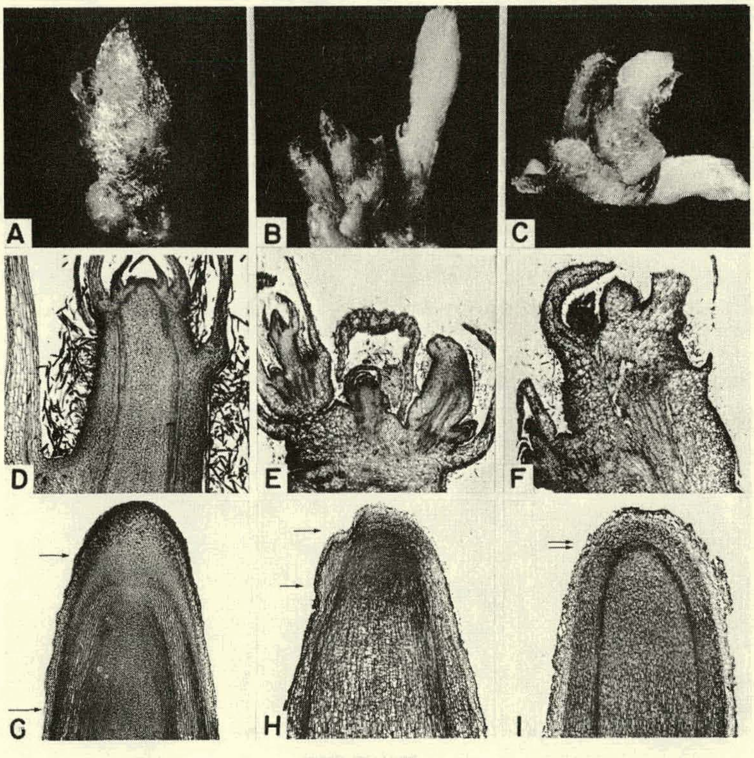


FIGURE 5

FIGURE 6. Relationship between radiation exposure and root growth at two 5-day intervals following germination. *Quercus alba* (A) had a slower inherent growth rate than *Quercus rubra* (B).

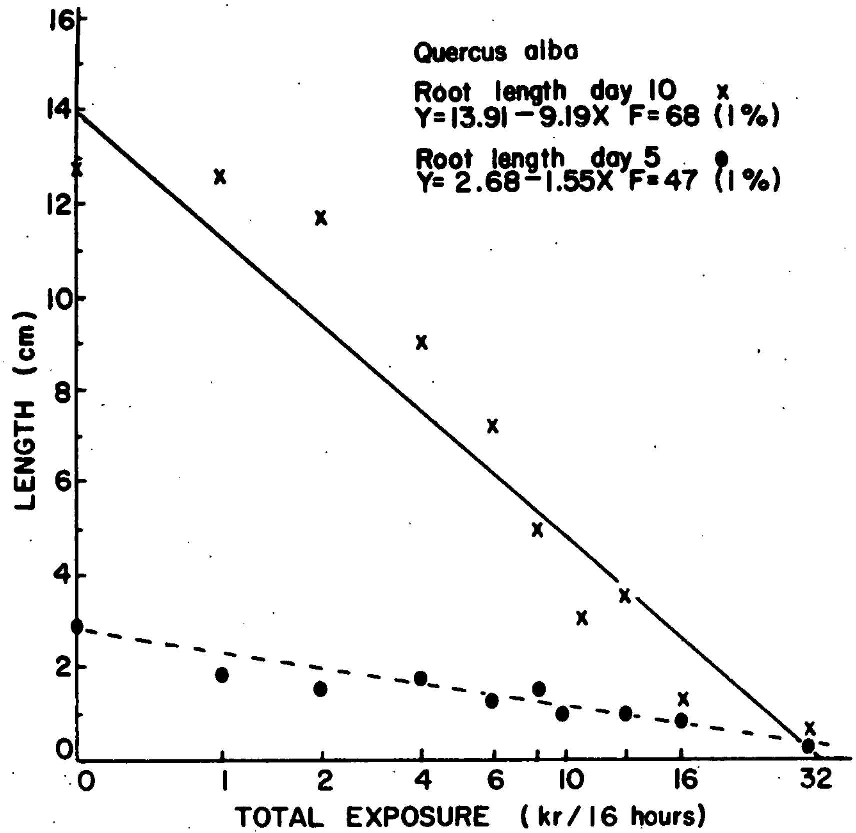


FIGURE 6A

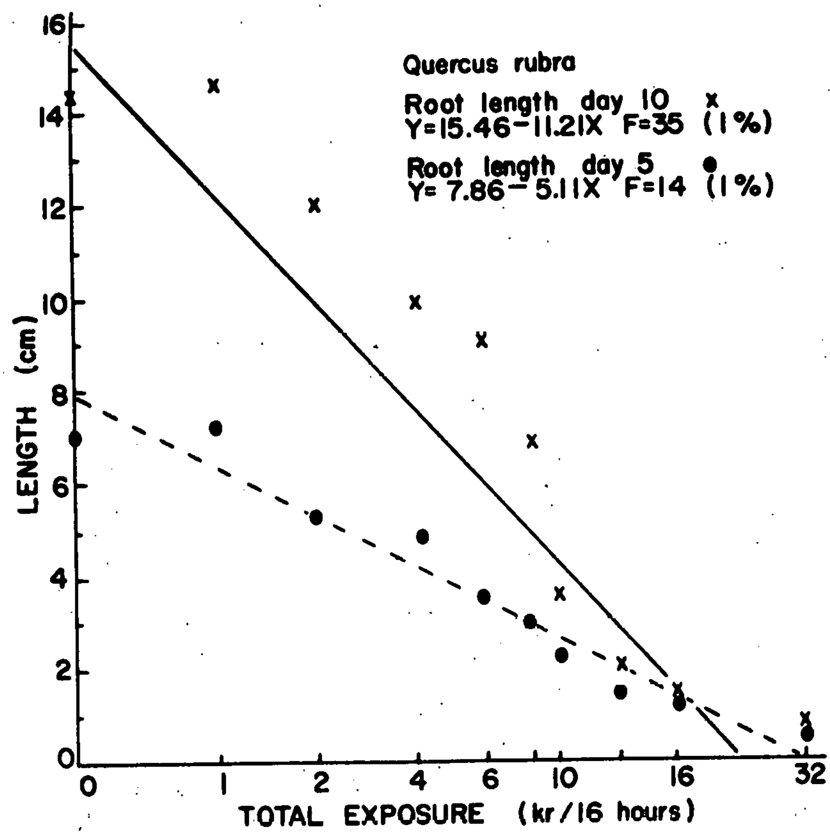
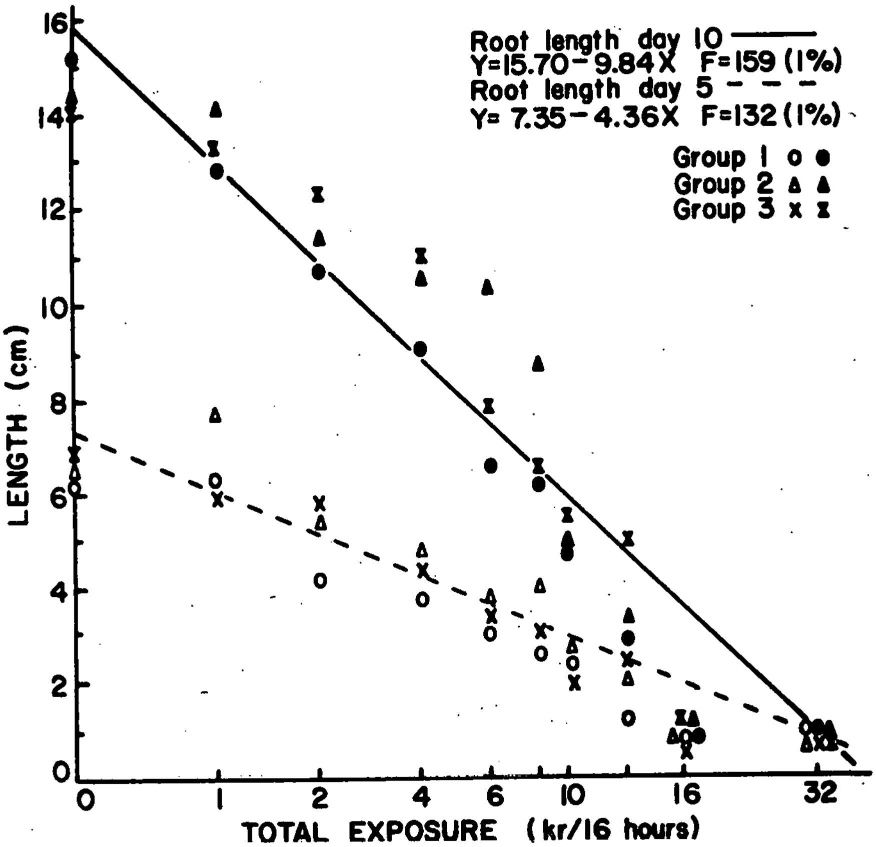


FIGURE 6B

FIGURE 7. Relation between root growth and radiation exposure of *Quercus rubra* seed for the following pre-irradiation conditions: Group 1, moisture content 22 percent stored at 4°C; Group 2, moisture content 44 percent stored at 4°C; and Group 3, moisture content 47 percent incubated 10 days at 29°C.



Root length day 10
Y=15.70-9.84X F=159 (1%)
Root length day 5
Y= 7.35-4.36X F=132 (1%)
Group 1
Group 2
Group 3
LENGTH (cm)
TOTAL EXPOSURE (kr/16 hours)
0
1
2
4
6
10
16
32

FIGURE 7