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Gamma and Fast Neutron Sensitivity of Tree Seed and Seedlings

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INTRODUCTION

Wolfe, Wareham and Scofield in their work on microclimates in 1949 noted:

> "The most critical portion of the atmosphere, as far as biota are concerned, is that next to the substrate itself. There environmental conditions are continually changing,- frequently at different rates at adjacent stations, as a result of differential loss of heat to air and space, and certain other differentials. These changes are most conspicuous in the lowest portion of that layer of air in which life occurs. This is the layer into which the new shoot emerges. What happens to the plant be it an annual jewelweed or the perennial redwood, during this critical period of its life determines whether it will live or die. The same can be said for other stages of development, but by and large the vicissitudes are most numerous and intense at the young seedling stage. Consider the 10-year survival probability of a 1-year old white oak plant with an individual of the same species at 250 years!" (62)

A differential of importance to the biota in the critical portion of the atmosphere, not considered by J.N. Wolfe and al. in 1949, is ionizing radiation. Homeostasis of any plant community is dependent upon the continuation of the same relative reproductive rates and survival values for the species inhabiting the area. Seed are the chief propagules of trees native to the deciduous forest. Thus, the effects of ionizing radiation on seed germination and seedling survival are of utmost importance. Relative seed sensitivity and factors affecting their sensitivity to specific types and combinations of ionizing radiations must be determined if we are to make a meaningful ecological approach to the problems associated with increased levels of ionizing radiation in the biosphere.

Early seed irradiation studies such as those by L. Ehrenberg et al. (17-18), R.S. Caldecott (7), C.F. Konzak (24-25), and R.A. Nilan (40-41) considered the effects of various factors that influence seed radiosensitivity of barley. The radiosensitivity of crop and horticultural plants was more widely investigated than the radiosensitivity of native species. Smith (50) thoroughly reviewed investigations conducted in search of mutants useful to man.

Not much was known of the relative radiosensitivity of native plant species or the factors affecting their sensitivity to gammas and fast neutrons when this series of investigations concerning tree seed and seedling radiosensitivity began in 1956. Seed of 18 tree species native to the Eastern Deciduous Forest were collected and exposed to acute doses of gamma radiation. Effects of gamma radiation on germination, seedling growth and survival varied from species to species, even within a given genus. Germination of seed of these 18 species exposed to 10kR ranged from 0 to 85 percent with 5 species less than 10 percent. Survival ranged from 0 to 82 percent with 10 species less than 10 percent. Seed of nine species germinated following 100kR but only two of these species survived past the seedling stage. In the garden, germination and survival are both less than they are in the laboratory, but the degree of variation ranges from slight to great depending on the species. Consequently, laboratory tests can not be used to predict with certainty what will occur in the garden or in native habitats where seed and seedlings are exposed to additional stresses.

Seed of many native tree species exhibit embryo or seed coat dormancy. *Quercus alba* L. seed germinates as soon as it ripens and is more radiosensitive than species with dormant seed. Seed of seven other species were irradiated while dormant and after dormancy was broken by stratification to determine whether physiological status affects radiosensitivity. Lower levels of gamma radiation are critical to physiologically active seed of these seven species. During stratification, seed water content is increased and water is known to affect sensitivity. To determine whether the increased radiosensitivity of physiologically active seed is the result of increased rate of metabolism or water content, water soaked and non-soaked dormant seed were irradiated. Increased water content did not increase the radiosensitivity of dormant *Juglans nigra* L. or *Quercus velutin* Lam. but did increase the radiosensitivity of physiologically active *Quercus alba* L. Consequently, the increased seed radiosensitivity following stratification is the result of increased physiological activity and water content rather than increased water content alone.

Seed samples were collected from a single tree or group of trees in the same area to reduce the effects of genetic variability from 1956 through 1959. In 1960 and 1961 seed samples of *Quercus alba* L., *Quercus velutina* Lam., and *Juglans nigra* L. were collected from Southeastern Kentucky, Northeastern Kentucky, and Southwestern Ohio to test the relative radiosensitivity of different populations of the same species. Germination and survival of the 3 populations of a given species did differ significantly. However, the relative radiosensitivity of the three species remains the same; *Juglans nigra* the most resistant and *Quercus alba* the most sensitive.

In preliminary investigations of the RBE of fast neutrons and gamma radiations on germination and survival of four species of tree seed, the species most sensitive to gamma radiation were relatively more resistant to neutrons than the most gamma resistant species. This indicates that these two types of radiation are not identical in their mechanisms of action. To determine whether fast neutrons and gammas are additive, the germination and survival of five species of tree seed exposed to 16 ratios of fast neutrons and gammas were recorded and analyzed. Significant neutron-gamma interactions indicate that these two forms of radiation are not additive over a wide range of environmental factors.

The following year soaked and non-soaked seed of four species were exposed to 16 combinations of fast neutrons and gammas. Water content, rate of physiological activity and interactions of these variables modify gamma and fast neutron effects differently; thus, these two forms of radiation have different modes of action. Soaked and non-soaked seed samples of four species were irradiated with four levels of gamma rays, and other samples of the same species with four levels of fast neutrons to determine whether the differences in neutron and gamma effects are the result of difference in water content. Increased water content reduced gamma sensitivity of 3 species and had no effect on one species at the levels tested. Increased water content increased neutron sensitivity of two species and had no effect on two species at the levels tested. Thus, gamma and fast neutrons do have different modes of action.

In the fall of 1967 three species of *Pinus* were planted as a biological check on the effectiveness of nuclear waste disposal at Maxey Flats, Kentucky. The great radiosensitivity of the gymnosperms makes the genus *Pinus* a desirable indicator of low level radiation stress. Analysis of seedling activity, observation of irradiation effects on seedling morphology, and monitoring seedling exposure doses over a period of time will make it possible to estimate the effectiveness of the nuclear waste disposal.

Juglans nigra L., *Fraxinus americana* L., and *Quercus alba* L. year old seedlings were irradiated while dormant in 1966 and other samples were irradiated as the buds broke in 1967, and planted in a secondary forest where the three species are native. The ability of irradiated seedlings of a given species to compete with one another and non-irradiated seedlings in an ecosystem exposed only to background irradiation is being investigated. On the basis of seedling survival and general vigor, ash is the most gamma resistant and white oak the most gamma sensitive of the three species.

Trees grown from control and irradiated seed are now bearing seed. Viability of seed that developed on X_1 generation trees under optimum conditions did not deviate from controls, but stress factors during seed development resulted in significantly lower germination. As seed develops on control and X_1 generation trees, the relative radiosensitivity is being tested to determine whether stress has a greater effect on the X_2 generation than the control. This study is being continued in an attempt to evaluate radiation stress as a selection pressure in the evolutionary development of a given species exposed to recurrent irradiation.

SEED RADIOSENSITIVITY RANGE FOR EIGHTEEN DECIDUOUS TREE SPECIES

Dormant seed of 18 tree species native to the Eastern Deciduous Forest were irradiated in 1956-57 with acute doses of cobalt-60 gamma rays. Seed samples of each species were collected from a single tree or group of trees in the same area to reduce the effects of genetic variability. Dormant seed samples were exposed to 0, 10, or 100kR gamma rays from a cobalt-60 source. Irradiation affected germination, seedling growth rate, and survival of each species in an individual fashion.

The critical level of irradiation is less than 10kR units of gamma rays for the following species that have less than 30 percent survival relative to controls at 10kR:

Acer saccharinum L.
Carya ovata Mill.
Nyssa sylvatica Marsh.
Pinus rigida Mill.
Quercus alba L.
Q. prinus L.
Q. velutina Lam.
Ulmus rubra Muhl.
Ulmus americana L.

The critical level of irradiation is between 10 and 100kR units of gamma rays for the following species that have greater than 60 percent survival relative to controls at 10kR:

Acer saccharum Marsh.
A. rubrum L.
Aesculus octandra Marsh.
Cercis canadensis L.
Fraxinus americana L.
Juglans nigra L.
Liquidambar styraciflua L.
Platanus occidentalis L.
Robinia pseudoacacia L.

The morphological effects of seed irradiation follow:

1. Germination is long delayed at 10kR.
   Carya ovata Mill.
   Quercus prinus L.

2. Inhibition of hypocotyl elongation results in cotyledons enlarging at soil surface and all seedlings dying before the first true leaf develops at 100kR.
   Acer rubrum L.
   A. saccharum Marsh.
   Cercis canadensis L.
   Robinia pseudoacacis L.
   Ulmus americana L.
   Nyssa sylvatica Marsh.

3. Inhibition of cotyledon enlargement at 10kR in *Pinus rigida* Mill. results in seedling death before the seed coat is shed.

4. Inhibition of growth of the epicotyl even though the roots develop extensively.
   Quercus alba L. - 10 and 100kR
   Q. prinus L. - 10 and 100kR
   Q. velutina Lam. - 50 and 100kR
   Aesculus octandra Marsh. - 50 and 100kR

5. Initial inhibition of internode elongation at 10kR.
   Acer rubrum L.
   A. saccharinum L.
   Cercis canadensis L.
   Nyssa sylvatica Marsh.
   Platanus occidentalis L.
   Quercus velutina Lam.
   Robinia pseudoacacia L.
   Ulmus americana L.

6. Failure of terminal buds to develop on many plants.
   Aesculus octandra Marsh. - 10kR
   Quercus alba L. - 5kR

7. First leaves are puckered, dissected and chlorotic sectors at 10kR.
   Acer rubrum L.
   A. saccharinum L.
   Aesculus octandra Marsh.
   Carya ovata Mill.
   Cercis canadensis L.
   Juglans nigra L.

The lower survival rate of seedlings from irradiated seed than from controls is related to the effects of irradiation noted above. Emergence may be great in some cases following seed irradiation, but inhibition of the growth of the epicotyl, hypocotyl, or cotyledons results in early death. The first true leaves of many seedlings that survive the initial stages of germination have scattered chlorotic sectors and are puckered or dissected. If the seedling survives this stage, the latter leaves are not affected. Seedling survival from this stage onward is about the same as that of controls. *Aesculus octandra* seedlings from irradiated seed grow more slowly than controls. This difference in growth rate persisted through the first 6 years of growth. *Cercis canadensis* seedlings from irradiated seed start out more slowly than controls but are as tall as controls at the end of the first year's growth. Seedlings from *Juglans nigra* seed irradiated with 5 or 10kR grew faster than the controls for 4 years, but in the fifth year the controls surpassed those from irradiated seed. *Liquidambar styraciflua* seedlings from seed irradiated with 10kR and 100kR start out more slowly than controls, but their growth rate increased and within 8 years were the same height as controls.

Variation in the radiosensitivity of members of a given plant community yields modified species survival values in irradiated communities. Living organisms are inseparably interrelated and interact upon each other. Thus, changes in species survival values would yield changes in magnitude as great or greater than those caused by wide spread diseases such as Chestnut Blight or Dutch Elm Disease.

EFFECTS OF PHYSIOLOGICAL ACTIVITY ON TREE SEED RADIOSENSITIVITY

Seed of many deciduous tree species lie dormant in the soil over winter or for several years before germination occurs. Consequently, seed could be irradiated over a long period of time if radioactive wastes were to accumulate in the soil or if the area were subjected to chronic radiation from any other source. Embryo dormancy is broken by storing seed stratified in moist sand at 0-5°C for the time period specific for the given species.

Quercus alba L. seed is more radiosensitive than the other species considered in previous tests. Since this species does not exhibit embryo dormancy, physiological status of seed was considered as a factor affecting radiosensitivity. Seed of 7 other species were irradiated both before and following stratification to test this hypothesis in 1957-58. The seed radiosensitivity of these species is greatly increased as the physiological activity increases. Much lower levels of gamma irradiation are critical once dormancy is broken. The critical range of gamma radiation in kR for dormant seed and physiologically active seed occurs in Table 1.

Table 1. Survival is greater than 60 percent of control at the lower level and between 0 and 25 percent of control at higher level of kR gamma radiation.

Dormant Seed	Species	Physiologically Active Seed
- - -	*Quercus alba* L.	1 - 5
5 - 10	*Carya ovata* Mill.	0 - 5
10 - 50	*Aesculus octandra* Marsh.	5 - 10*
10 - 50	*Cercis canadensis* L.	0 - 5
- - -	*Fraxinus americana* L.	10 - 50
10 - 50	*Juglans nigra* L.	5 - 10*
10 - 50	*Liquidambar styraciflua* L.	5 - 10
10 - 50	*Platanus occidentalis* L.	0 - 5

* Survival between 40 and 49 percent of control at higher level.

Lower levels of gamma radiation are more critical to the survival of physiologically active tree seed than to dormant tree seed. Morphological effects are also more pronounced and occur at lower levels when irradiation follows stratification (Table 2).

Table 2. Summary of effects of seed irradiation before and after stratification.

Species	Seed irradiated before stratification	Seed irradiated after stratification
Aesculus octandra Marsh.	Average height in inches Control 9.9 5kR 8.2 10kR 5.4	Average height in inches Control 9.6 5kR 6.4 10kR 1.9
	Plants with terminal buds Control 100% 5kR 40% 10kR 10%	Plants with terminal buds Control 100.0% 5kR 19.0% 10kR 4.3%
Cercis canadensis L.	Cotyledons enlarge at soil surface - 50kR	Cotyledons enlarge at soil surface - 5 and 10kR No germination - 50kR
	Growth rate same as control - 5 and 10kR	Growth rate slower than control - 5 and 10kR
Juglans nigra L.	Rate of elongation same as control - 5 and 10kR	Rate of elongation 1/5 that of control - 10kR
Liquidambar styraciflua L.	Germination high in relation to control 5 and 10kR	Germination and survival very low at 10kR and zero at 50kR
	Low germination and survival - 100kR	
Platanus occidentalis L.	Rate of stem elongation and leaf enlargement at 5 and 10kR not as rapid as control	Germination and survival very low at 5 and 10kR

The water content of seed increased during stratification; thus, the water content of stratified seed is higher than that of dormant seed at the time of irradiation. Because water does modify radiosensitivity, these investigations of the comparative radiosensitivity of seed before and after stratification raise the question of whether the differences are a direct effect of water content or are associated with the degree of physiological activity.

EFFECT OF WATER CONTENT ON RADIOSENSITIVITY

Dormant seed of *Juglans nigra* L. and *Quercus velutina* Lam. have low rates of physiological activity at both high and low water contents until embryo dormancy is broken. The seed of *Quercus alba* L. is physiologically active as soon as it ripens. If increased radiosensitivity of hydrated seed is the result of increased physiological activity, soaked and non-soaked dormant seed would have the same radiosensitivity.

Seed samples of the above 3 species were soaked in water at room temperature for 48 hours and then irradiated along with non-soaked samples at the UT-AEC Agricultural Research Laboratory. Seed water content at the time of irradiation is determined by drying at 100°C for 48 hours and calculating the percent water on the basis of wet weight. *Quercus alba* seed was planted following irradiation. *Quercus velutina* and *Juglans nigra* seed were stratified in moist sand at 0 - 5°C for 45 and 120 days respectively preceding planting. Germination and seedling survival both having ecological significance were used as a measure of relative radiosensitivity in this study. The significance of variations in germination and survival of these two treatments were tested by an analysis of variance.

Increasing the water content of physiologically active *Quercus alba* seed from 44.5 to 49.4 percent in 1959-60 did increase their radiosensitivity significantly. The radiosensitivity of dormant *Quercus velutina* and *Juglans nigra* seed was not significantly altered when the water content was increased as follows:

Quercus velutina	1959-60	30.8	to	37.6	percent
Juglans nigra	1959-60	10.1	to	26.2	percent
J. nigra	1960-61	22.3	to	38.1	percent
J. nigra	1961-62	30.7	to	39.7	percent

In 1957-58 the radiosensitivity of 6 tree species with dormant embryos was greatly increased when the seed became physiologically active. The seed water content increased as dormancy was broken during stratification. Increasing the water content of dormant seed did not alter their radiosensitivity as it did physiologically active *Quercus alba* seed. Therefore, the increased seed radiosensitivity following stratification is the result of the interaction of increased physiological activity and water content rather than increased water content alone.

RADIOSENSITIVITY OF DIFFERENT GENETIC POPULATIONS OF A GIVEN SPECIES

Seed samples of each species investigated from 1956 through 1959 were collected from a single tree or group of trees in the same area to reduce genetic variability to a minimum. To determine whether the conclusions concerning the effects of seed irradiation of each species tested through 1959 could be applied to all members of the species or to no more than the one genetic population sampled, seed samples of *Juglans nigra* L., *Quercus alba* L., and *Q. velutina* Lam. were each collected from different genetic populations and the radiosensitivity of each population was determined and compared with each of the others of the same species.

Seed samples of each species were collected from a single tree or group of trees in the following areas:

(1) Letcher County, Southeastern Kentucky
(2) Rowan County, Northeastern Kentucky
(3) Butler County, Southwestern Ohio

Juglans nigra seed samples were collected in each of these areas and irradiated with 0, 5, 10, 15, 20, 30kR in 1960 and 0, 5, 10, 15, 20, 25kR units of gamma rays in 1961. *Quercus alba* seed samples were collected in NE Kentucky and SW Ohio in 1960 and irradiated with 0, 2, 4, 6, 8, 10kR units of gamma rays. Seed samples of *Quercus velutina* were collected in SE Kentucky and SW Ohio in 1960 and irradiated with 0, 5, 7, 10, 15kR units of gamma rays. No other viable seed samples were collected in either 1960 or 1961.

A statistical analysis of the data collected from the tests described above was made with an analysis of variance. A summary of the significance of the deviation between population follows.

Species and Collection Counties	1960 Germination	1960 Survival	1961 Germination	1961 Survival
Juglans nigra L.				
Rowan-Letcher	N.S.	**	**	**
Rowan-Butler	N.S.	*	**	**
Letcher-Butler	**	**	**	**
Quercus alba L.				
Rowan-Letcher	--	--	**	**
Rowan-Butler	N.S.	*	**	**
Letcher-Butler	--	--	N.S.	N.S.
Quercus velutina Lam.				
Letcher-Butler	**	**	--	--

* Significant
** Highly Significant
N.S. Not Significant
-- Seed Not Available

Although there are differences in the degree of radiosensitivity from one genetic population to another, irradiation effects on a given species follow the same general pattern. In all three areas *Quercus alba* had the greatest radiosensitivity and *Juglans nigra* the lowest radiosensitivity of the three species investigated. Consequently, the data obtained from seed samples of a given species collected in Rowan County, Kentucky and used in various experiments in past years can be applied in a general fashion to the species as a whole.

The significant variations in average germination and survival between genetic populations is in contrast to the non-significant variation in germination and survival of *Juglans nigra* L. seed collected in Rowan County in 1960 and 1961, Letcher County in 1960 and 1961, or Butler County in 1960 and

1961. Environmental factors were not the same during the 1960 and 1961 growing seasons in any of the 3 collection areas. However, the variations in germination and survival from year to year for a given species from a given area were not significant. This indicates that the significant deviations between populations of a given species are at least partially genetic in origin.

RELATIVE RADIOSENSITIVITY OF DECIDUOUS TREE SEED TO FAST NEUTRON AND GAMMA RADIATIONS

Effects of gamma radiation on deciduous tree seed and herbaceous species vary from species to species, even within a given genus (21, 52, 60). Konzak (26) lists the following factors that modify radiation effects in cells: genotype, stage of nuclear cycle, water content and physiologic state, seed storage, seed age, elements present, chemical treatments, kinds of radiation, dose rate, temperature, and atmosphere. Each of these factors has been investigated by numerous workers from varied points of view.

The water radical prevents (8, 16, 24) while the oxygen radical enhances (1, 8, 42) post-radiation induced biological damage, and both modify post-radiation seed storage effects (8,39, 40). Nuclear volume and DNA content are also correlated with plant tolerance to chronic radiation (5, 55). Increased nuclear volume without change in chromosome number increases radiosensitivity, but increased chromosome number without a change in nuclear volume has a protective effect. Relative radiosensitivity is defined by two variables - nuclear volume and chromosome number (19, 54). Sparrow and Woodwell (56) predict sensitivity of plants to chronic gamma irradiation on the basis of nuclear and environmental factors affecting radiosensitivity.

Zimmer (68) explained biological damage and modification of this damage on the basis of production and survival of radiation induced free radicals that act as intermediates between energy absorbed and the observed final effect. Energy rich free radicals exist longer in systems with restricted mobility (low temperature or low water content) and oxygen combines with free radicals yielding more reactive radicals. Thus, both decreased water supply and increased oxygen content yield greater biological damage (11). Densely ionizing radiation yields lower concentrations of free radicals per dose unit, for apparently most of the induced radicals are eliminated by recombination because of their close proximity (15).

The relationship between the dose of a standard type of radiation and the dose of the tested type of radiation that gives an equivalent biological effect is expressed as the relative biological effectiveness, RBE. The RBE of x-rays, gamma rays, fast neutrons and thermal neutrons may vary over wide limits depending upon the aberration under consideration, the material being irradiated, the physical environment preceding, during and following irradiation (3, 8, 12, 18, 20, 24, 30, 31).

Although seed radiosensitivity of barley and a few other cultivated species have been investigated intensively, relatively few studies of native tree species (21, 22, 35, 36, 43, 51, 60) have been undertaken. Native tree species have a more variable environment and are genetically more heterozygous than commercial varieties. Although seed production of native species is irregular, one good crop every 2, 4, or 6 years can account for their survival but the elimination of a crop variety. Seed of many species native to the temperate region often do not germinate the year following seed dispersal because they have dormant embryos and/or resistant seed coats. Genetic variability and dormancy make native tree seed radiosensitivity ecologically interesting and important to an understanding of total effects of irradiation in a given community.

Seed of the following species were collected as they ripened in 1962 from the same genetic population samples in previous studies to eliminate genetic variability as a source of error in making comparisions.

Fraxinus americana L. | *Juglans nigra* L.
Liquidambar styraciflua L. | *Platanus occidentalis* L.

Physiologically active seed samples of each of the above species and dormant seed samples of the latter 2 species were irradiated. Seed samples of each of the above species were exposed to 877, 1755, 3510 and 7020 rads of neutrons in the West Animal Tunnel of the ORNL Graphite Reactor in a boron carbide impregnated plastic box that eliminated the thermal flux. Other samples of the first 3 species were exposed to 10, 20, 30 and 40kR and the latter species to 5, 10, 15 and 20kR of gamma rays at the UT-AEC Agricultural Research Laboratory's cobalt-60 source.

The relative biological effectiveness (RBE) of neutrons was determined by dividing the level of gamma radiation that yields a given effect by the level of neutrons that produces the same effect. Seed germination and seedling survival are used as a measure of radiosensitivity.

The RBE of neutrons varies from species to species and level to level of radiation. When the RBE equals 1, the two types of radiation have the same effect at the same level. The higher the RBE the greater the sensitivity to neutrons in comparison with gamma rays. The RBE range for physiologically active seed irradiated at the levels noted above is as follows:

Species	RBE range for germination	RBE range for survival
Liquidambar styraciflua L.	11.4 - 5.7	5.7 - 5.7
Fraxinus americana L.	11.4 - 8.6	5.7 - 4.3
Juglans nigra L.	5.7 - 4.3	5.7 - 4.3
Platanus occidentalis L.	5.7 - 2.8	5.7 - 4.3

The gamma sensitivity ranges from slight to great in the following order: *Liquidambar styraciflua* L., *Fraxinus americana* L., *Juglans nigra* L., *Platanus occidentalis* L. On the basis of the experiments noted in this section, neutron sensitivity is the inverse of gamma sensitivity. Note that the RBE of neutrons is higher for the species more resistant to gamma rays than for more gamma sensitive species.

In these preliminary investigations of RBE of fast neutron and gamma radiations on germination and survival of tree seed, the species most sensitive to gamma radiation were relatively more resistant to neutrons than the most gamma resistant species. Zirkle (69) noted that complete additivity of two types of radiation indicates that the mechanisms of action of the radiations are identical in their most essential feature, the promotion of the same determinative events but not necessarily alike otherwise; and that incomplete additivity indicates differences in mechanisms of action. To determine whether fast neutron and gamma radiations are additive, the germinations and survival of tree seed exposed to 16 ratios of fast neutrons and gammas were recorded and analyzed.

The effects of oxygen, water content, free radicals, nuclear volume, rate of metabolism, storage, and age of seed on radiosensitivity have been studied in detail under controlled environmental conditions (8, 11, 17, 25, 27, 28, 29, 42, 44, 49, 53, 63). Four of the 5 species considered in the present investigation have dormant embryos that become physiologically active following stratification in moist sand at 0-5°C for 60-120 days. Seed water content, rate of metabolism and nuclear volume increase as the quantity of free radicals and mobility of oxygen decrease in the system during stratification. Physiologically active seed has a greater water content, rate of metabolism and nuclear volume than dormant seed at the time of irradiation; but the oxygen content is lower. The effects of each of these factors are known to vary with the type of radiation. Native species thus compound the problems of radiosensitivity, but it is impossible to estimate radiation effects at the community level unless such complex interactions are investigated. Increased water content reduces oxygen mobility and favors free radical interactions that result in decreased storage gamma effects (11). The effectiveness of fast neutrons is reported to be independent of seed water content in barley (16), oxygen pressure (17, 20), storage effects (12), and physiological conditions of cells (13). Increases in nuclear volume during mitosis (56) and preceding active spring growth in woody species (57, 61) result in increased radiosensitivity (19, 54).

Dormant and active walnut and sycamore, active ash and white oak and dormant sweet gum seed samples were exposed to 4 levels of fast neutrons, then each of these to 4 levels of gamma rays. Germination and survival of the 4 replicas of each species exposed to these 16 combinations of neutrons and gammas were analyzed. The gamma effect on germination and survival was modified by the level of neutrons in 10 of the 12 tests listed above. The non-significant neutron-gamma interactions in the other two tests were the result of exposing the seed to levels of radiation too low to affect germination. The significant neutron-gamma interactions indicate that the effects of these two types of radiation are not additive in either dormant or physiologically active seed. This indicates that these 2 forms of radiation are not additive over a wide range of environmental factors. The relative effects of physiological activity on radiosensitivity were not determined because a miscalculation resulted in fast neutron dose levels differing for dormant and active seed samples.

In 1964-65 soaked and non-soaked samples of dormant ash, sycamore, walnut and physiologically active white oak seed were exposed to 16 different combinations of fast neutrons and gammas. Neutron-gamma effects on germination and survival of these 4 species are not additive for the gamma effects on soaked and non-soaked seed samples were modified by the level of neutrons. The gamma effect was modified by the water content in each species except walnut; however, the modification of the neutron effect by water was variable from species to species. The water effect on gamma sensitivity was modified by the level of neutron irradiation, high rate of metabolism in white oak seed at the time of irradiation, and the low temperature storage period following irradiation in sycamore, walnut and ash. The combination of increased water content and low temperature storage of dormant ash and sycamore seed resulted in a decrease in their sensitivity to various ratios of fast neutrons and gamma radiations, but the effect on dormant walnut was variable from level to level. High water content increased the sensitivity of physiologically active white oak seed to the ratios of fast neutrons and gammas tested. Water content, rate of physiological activity and interactions of these variables modify gamma and fast neutron effects differently; consequently, these two forms of radiation have different modes of action.

In 1963-64 and 1964-65 investigations, seed samples were exposed to 16 combinations of fast neutrons and gamma rays to determine whether these two types of radiation are additive or independent in action. The significant neutron-gamma interactions are evidence that the neutron effect is conditioned by the level of gamma rays and that fast neutrons and gammas are not additive. Thus, these two types of radiation are inferred to have different modes of action. In the analysis of variance of both percent germination and survival and arc-sin transformations of these percentages, the seed water content modified the gamma effect, but had variable actions on the neutron effect. Soaked and non-soaked seed samples of each species were irradiated with 4 levels of gamma rays, and other samples of the same species with 4 levels of fast neutrons in 1965-66 to determine whether the differences in neutron and gamma effects are the result of differences in water content. Increased water content reduced gamma sensitivity of dormant walnut, ash, and sycamore, but had no effect on dormant sweet gum at the levels tested. Increased water content increased neutron sensitivity of ash and sweet gum, but the effects were not significant on sycamore or walnut seed. The gamma and fast neutron sensitivity of physiologically active white oak seed was slightly increased by soaking the seed in water preceding irradiation.

Increased water content decreased gamma sensitivity of dormant seed by decreasing mobility of oxygen and increasing union of free radicals without increasing mitosis, rate of metabolism, or nuclear volume. Neutron effects are magnified by hydrogen or proton recoils and heavy atom recoils. Heavy atom recoils have higher LET (linear energy transfer) than do proton recoils (20). Increased water content thus decreases the LET. Sparsely ionizing gamma rays yield a low density of free radicals, but neutrons leave a dense track of free radicals. Increased water content increases the mobility of the sparse free radicals induced by gamma radiation and approximately 90 percent of them unite yielding the original molecule (20). This reaction results of decreased gamma sensitivity. Increased water content also increases the mobility of the dense track of free radicals induced by neutron radiation; however, movement of dense free radicals in close proximity results in abnormal products and increased biological damage (20). Increased water content at the time of irradiation increases the mobility of the induced free radicals, however, the results of this increased mobility is dependent upon the density of the free radicals. In some cases the increase in free radical mobility is not great enough to yield different effects in soaked and non-soaked dormant seed. Increased water content of physiologically active white oak seed not only affects the union of free radicals, but also increases nuclear volume, mitosis, and rate of metabolism. Consequently, both fast neutron and gamma sensitivity of white oak seed are increased by soaking the seed preceding irradiation.

Increasing the water content of dormant ash seed decreased their gamma sensitivity and increased their sensitivity to fast neutrons in the 1966 investigations. The excess stratified, soaked, and non-soaked irradiated seed samples were stored at 0-5°C. The soaked seed samples decayed but non-soaked samples that were still in good condition 12/68 were stratified and planted 5/69. Germination and survival of these four 100 seed samples planted 5/69 were lower than those planted 6/66. However, the same general patterns of radiosensitivity occurred in 1966 and 1969. At the levels tested, germination and survival of the neutron irradiated non-soaked seed were greater than they were for gamma irradiated seed. Survival of the gamma irradiated seed was 0.0 percent as it was in 1966 and ranged from 37 to 0.0 percent at the neutron levels tested. One stratified sample of seed irradiated at each level of gammas and fast neutrons 12/65 was stored at 0 - 5°C from 1/66 until planted 4/69. Although germination and survival of these seed samples were much lower than those planted in 1966, increased water content continues to increase germination and survival of gamma irradiated seed and decrease germination and survival of neutron irradiated seed.

The effects of water on gamma and fast neutron sensitivity are not short term effects but persist during three years of stratification or three years of dry storage at 0-5°C. Thus, it is evident that increased water content decreases gamma sensitivity and increases neutron sensitivity of dormant ash seed. Each of the experiments described in this section support the hypothesis that fast neutrons and gammas are not additive in action and that they do have different modes of action.

PINE SEEDLINGS AS A BIOLOGICAL CHECK ON THE EFFECTIVENESS OF NUCLEAR WASTE DISPOSAL AT MAXEY FLATS, KENTUCKY

The great radiosensitivity of the gymnosperms makes the genus *Pinus* a desirable indicator of low level radiation stress. Sparrow, Rogers, and Schwemmer (59) predict the LD50's for gymnosperms to be in a range from 460-1200 R of acute gamma radiation on the basis of interphase chromosomal volume. Plant communities under chronic irradiation whether they be old field communities (66) or near climax forest (65) had even greater sensitivity than expected when the entire ecosystem was irradiated and the plants were exposed to new environmental stresses. *Pinus rigida* bordering the Brookhaven National Laboratory's (BNL) gamma field for 10 years was injuried by exposure doses as low as 5k/20 hr day (58). *Pinus monophylla* stem elongation was inhibited by doses as low as 15 R (6). Chronic gamma irradiation of *Pinus rigida* and *P. strobus* result in severe inhibitation of growth and ultimate death of some trees at daily exposure rates of 6 R/day and lower. The reduced growth of branches and needles and premature shedding of needles yielded a greatly reduced amount of photosynthetic tissue, resulting in sparse crowns and low food synthesis that lead to starvation (4). Mergen and Thielges (37) also noted that irradiation decreased *Pinus rigida* stem elongation and that flushing was delayed while sprouting increased and needle length decreased. Actively growing *Pinus strobus* seedlings investigated by Platt (47) were more sensitive than dormant seedlings around the air shielded nuclear reactor in the hills of Georgia. Sparrow et al. (54) relate increased radiosensitivity of actively growing *Pinus strobus* to increased nuclear volume. Capella and Conger (10) note a correlation between radiosensitivity and interphase chromosomal volume of 5 gymnosperm species whose LD50's range from 500-800 R. Necrosis of vegetative buds and plant death in *Pinus pinea* and *Pinus halepensis* were the result of mitotic inhibitation of apical and subapical meristem cells (14). Woodwell et al. (67) report that doses greater than 2 kR slow or divert succession toward a less complex climax in coniferous forests.

Because the gymnosperms exhibit a number of morphological aberrations at comparatively low levels of radiation, three species of pine were planted in 1967 over nuclear waste disposal pits ten miles north of Morehead State University as a long term biological check on burial methods being employed. Nuclear Engineering Inc. deposits nuclear wastes in parallel trenches 300 feet long, 50 feet wide, and 50 feet deep. Levels per pit would measure in mega curies if wastes were not shielded. Wastes are buried in a single trench until it is filled and closed over with soil. Readings as high as 50 mR/hr occur in the area around an open pit, but maximum readings over a closed pit are no greater than 10 to 20 mR/hr.

Equal numbers of *Pinus echinata* Mill., *P. strobus* L., and *P. virginiana* L. were planted in a random pattern over four nuclear waste burial trenches and a control area in 1967, a total of 216 seedlings per plot. Each year the seedlings are measured, morphological aberrations are noted, the exposure dose of representative seedlings is monitored for 6 to 9 weeks during the growing season, and the radioactivity of specimens of each species from each experimental plot is analyzed. The radioactivity of seedlings growing over the burial pits from the winter of 1967 until the summer of 1971 was no greater than controls. Thus, in this length of time there hasn't been a greater movement of nucleutides into the experimental trees than the controls.

Exposure doses over the burial pits range from two to 12.6 times those of controls, but they are still relatively low, none greater than 5.3m rem per day. The first morphological damage occurred during the 1970 growing season; reduced internode elongation resulted in tuffs of needles at the stem apex of some seedlings, and premature shedding of needles.

During the winter and spring of 1971-72 all the burial pits were covered with an additional 3 feet of soil. All experimental trees were killed. New plantings were made in the summer of 1972. Analysis of seedling activity, observation of irradiation effects on seedling morphology and survival, and monitoring seedling exposure doses over a period of time will make it possible to estimate the effectiveness of nuclear waste disposal methods employed by Nuclear Engineering Inc. at Maxey Flats, Kentucky. We shall continue this project with University research funds.

LONG TERM STUDY OF TREES GAMMA IRRADIATED AS SEED OR SEEDLINGS AND PLANTED IN A SECONDARY FOREST

Radiation ecology is not radiation effects on organisms but effects on a species and its functional position in a community as Platt pointed out in 1962 (45). In the first year of the Brookhaven experiment on the effects of ionizing radiation on a terrestrial ecosystem, plants were more sensitive than anticipated on the basis of chromosomal number and volume (64). Woodwell and Sparrow (65) cite environmental stress caused by the alteration of the environment as the cause of increased sensitivity. Beatly (2) investigating the winter annual vegetation following a nuclear detonation at the Nevada Test Site notes that the possible effects of ionizing radiation may be obscurred by those of other variables. Hardwoods exposed to acute radiation from an air shielded nuclear reactor in the N. Georgia exhibited: (1) an early leaf fall and almost complete inhibitation of leaf production the year following 12 to 15 krad; (2) prolongation of dormancy proportional to dose; (3) terminal buds killed and aberrant leaves at 3 to 4 krad; (4) leaf production cut 1/2 to 1/3 by 4 to 5 krad (46). Oaks, in the forest surrounding the BNL Cobalt Field, exposed to chronic radiation for ten years had sparse foliage, short internodes, few large distorted leaves, reduced number of flower primordia and reduction of viable pollen (38). In each of the cases noted, the entire ecosystem was irradiated, each organism exposed to a new stress, and individuality was expressed as it is for other stresses.

The success of any species in the ecosystem is dependent upon its ability to complete with members of the same and other species. Irradiation effects on growth rates and growth patterns will have an effect on competition. Seedlings exhibited greater sensitivity than expected when the whole ecosystem was irradiated (66). In the present investigation, the ability of irradiated seedlings of a given species to compete with one another and non-irradiated seedlings in an ecosystem exposed only to background irradiation was investigated in an attempt to gain insight into an understanding of population dynamics.

Juglans nigra L. (black walnut), *Fraxinus americana* L. (white ash), and *Quercus alba* L. (white oak) seed were collected fall 1965 from the same seed sources used in earlier seed irradiation experiments. Following stratification, the seed was planted spring 1966. Dormant seedlings during the winter of 1966-67 and physiologically active seedlings in spring of 1967 were gamma irradiated at appro· ımately 0, 3, 6, 9, 12kR. Entire seedlings were irradiated with dose level varying less than 500 R from tip of stem to tip of root. A random planting chart was made out for each species and then a seedling irradiated in the fall and spring at the same level were planted side by side. This was done so that comparisions between Fall and Spring irradiated seedlings would not be complexed by environmental differences.

Other than late spring frosts, growing conditions have been favorable since the seedlings were planted in 1967-68. The walnut buds break open earlier on young seedlings than they do on mature trees and are frequently severly damaged by late frosts. Such injury to the other two species is slight.

On the basis of seedling survival and general vigor, ash is the most gamma resistant and white oak the most gamma sensitive of the three species studied. Survival and stem elongation decrease as the level of irradiation increases. Ash and white oak are both gamma sensitive when physiologically active. Sixteen or more of the 20 dormant ash seedlings exposed to doses as great as 8.6kR survived and continue to grow through 1972, but fewer than 1/5 of the physiologically active seedlings survived doses of 5.9kR or greater. White oak, a more sensitive species, did not survive radiation doses as great as 5.5kR while physiogically active, and only 1/10 of the dormant seedlings survived 5.6kR. Walnut seedlings appear to have greater radiosensitivity while dormant than while actively growing. However, the greater viability of spring irradiated walnut seedlings is the result of root sprouts that arise because the auxin content decreases when the leaves die following irradiation and frost damage. The fall irradiated walnut seedlings were not so severely damaged early, began to grow, and most of the food reserves were used before death of the above ground parts occurred. Consequently, sprouting did not occur as frequently in the fall irradiated seedlings.

Sparrow et al. (54) report that nuclear volume of plants in active growth is about 1.5 time their volume when dormant. Increased nuclear volume without change in chromosome number increases radiosensitivity (19, 54). Interphase chromosomal volume has been found to correlate with radiosensitivity more precisely than nuclear volume (10, 59). The increased radiosensitivity of physiologically active seedlings would thus be expected. Ash seedlings irradiated while physiologically active not only had a lower survival value, but those that survived have grown more slowly than those irradiated while dormant. During 1968 and 1969 the growth rate of walnuts was greater in those irradiated while physiologically active. This is the result of the change in their growth pattern. Sprout elongation is much more rapid than the elongation of the main axis of a tree seedling. White oak has grown so slowly that stem elongation is not measurable.

The mean increase in length of both ash and walnut were relatively slight the first growing season, had a comparatively rapid increase the second season, and now that the trees have begun to branch out the terminal increase in length has decreased.

The changes in irradiated island "communities" from granite out-crops studied by McCormick and Platt were the result of differences in radiosensitivity of species (33). The variations in survival and growth rates among gamma irradiated ash, walnut, and white oak seedlings planted in a secondary forest are evidence that an irradiated ecosystem inhabited by these three species would change as a result of differences in species radiosensitivity. The environmental stresses caused by alterations of the environment due to differential species survival will effect competition and result in additional species variation within the ecosystem.

Seedlings grown from irradiated seed were transplanted in 1959 to: (1) a pasture at the UT-AEC Agricultural Research Laboratory's farm in Oak Ridge, Tennessee; (2) a secondary forest stand in the Cumberland National Forest in Morehead, Kentucky. Just as Woodwell and Sparrow (65) found plants irradiated within an ecosystem more sensitive than expected, the seedlings from irradiated seed are growing more vigorously and reproduction occurs at an early age in the pasture where competition has been reduced by spacing plants, keeping the pasture mowed and occasional fertilization than they are in the secondary forest stand. Comparative studies of these two communities provides data on the effects of competition on growth, reproduction, and survival of species exposed to irradiation as seed. Germination and survival of irradiated seed in the laboratory are also greater than they are in the field where the environmental factors are more severe.

EFFECTS OF GAMMA RADIATION OF TREE SEED ON THE RADIOSENSITIVITY OF THE X_2 GENERATION

Three hundred twelve seedlings grown from irradiated and non-irradiated seed of buckeye, red bud, white ash, black walnut, sweet gum, sycamore, white oak, and black oak were transplanted April 1959 to the UT-AEC Agricultural Research Laboratory's Farm, Oak Ridge, Tennessee. Germination and survival of the first seed crop (1965) of ash and red bud from X_1 generation trees did not differ significantly from controls. However, following poor seed production in 1967, the germination and survival of the 1967 X_2 red bud seed were significantly lower than were the seed from controls. Viability of seed that developed on X_1 generation trees under optimum conditions did not deviate from controls, but stress factors during seed development resulted in significantly lower germination. Rudolph (48) noted that germination of Jack pine seed exposed to 1 kR was greater than controls, but survival at the end of the year was but 30 percent of the control and their reproductive potential was reduced to 5 percent. He concluded that at least two generations are required to fully evaluate the effects of seed irradiation.

In previous experiments, the radiosensitivity of walnut seed collected from populations in South Eastern Kentucky, North Eastern Kentucky, and South Western Ohio, differed significantly. Morphologically indistinguishable seed and seedlings of different races of *Sedum pulchellum* were separated by McCormick (32) on the basis of differences in radiosensitivity. Such differences in radiosensitivity from population to population or within a population of a given species could be an important population control factor.

Campbell (9) reported that decreased barley seedling height in the 2nd generation of recurrent irradiation was probably the result of increased genetic load which was followed by elimination of aberrant cells and a third generation increase in size. He concluded that recurrent irradiation in consecutive generations to increase and retain the frequency of mutations was not feasible because of high level discrimination against induced mutation. McCrory and Grun (34) noted that radiation damage to potato seedlings was greater in plants whose ancestors had received a comparatively high dose of radiation. They also attributed the increased damage to an increased genetic load. However, Joshi and Frey (23) made successful genetic gains in oat seed weight by alternating several cycles of mutagen treatment and selection.

The reduced quantity of viable seed from the X_1 generation of red bud (1969 experimentation), Jack pine (48), barley (9) and potatoes (34) is evidence that radiation could be a powerful population control mechanism. This series of investigations by independent workers again demonstrates the difficulties of formulating ideas of radiation effects from short term experiments.

Ash seed production was great enough in 1969 to continue irradiation of seed from X_1 generation trees grown from seed exposed to 0, 5, 10kR gamma rays while dormant or physiologically active. Germination and survival of X_2

generation ash seed exposed to 0, 10, 20, 25, 30kR gamma rays did not differ significantly from control seed exposed to the same levels of gamma radiation.

As seed develops on control and X_1 generation trees of the eight species growing at the UT-AEC Research Farm in Oak Ridge, Tennessee the relative radiosensitivity is being tested to determine whether stress has a greater effect on the X_2 generation than the control as it did on the 1967 red bud seed. If the X_2 generation seed is not able to withstand stress as effectively as controls, seed irradiation could be an important population control factor for longer than a single generation. These studies are being continued in an attempt to evaluate radiation stress as a selection pressure in the evolutionary development of a given species exposed to recurrent irradiation.

SUMMARY

Genetic variability and dormancy make native tree seed and seedling radiosensitivity ecological interesting and important to an understanding of the total effects of irradiation in a given community.

1. The seed gamma sensitivity of 18 tree species varied from species to species, even with a given genus. The effects of acute gamma irradiation of seed are as follows:
   (A) Germination does not occur or is long delayed.
   (B) Emergence may be great, but inhibition of epicotyl, hypocotyl or cotyledon growth results in early death.
   (C) The first true leaves may be irregular, however, if the young seedling survives the latter leaves are not affected.
   (D) Growth of seedlings from irradiated seed may be slower than controls for as long as seven years.
2. Seed of tested tree species with dormant embryos were more radioresistant than species exhibiting no dormancy. When seed dormancy of seven species was broken before irradiation, lower levels of gamma radiation were critical to survival and morphological effects were more pronounced.
3. Dormant tree seed have low rates of physiological activity at both high and low water contents. Physiological activity and water content both increase as seed dormancy is broken. Increasing the water content of dormant seed

samples of two species before irradiation did not increase their gamma sensitivity. Thus, increased physiological activity rather than increased water content alone accounts for increased radiosensitivity of seed irradiated after dormancy is broken.

4. Both germination and survival of seed samples of *Juglans nigra* L. collected from three different populations differed significantly from population to population. However, niether germination or survival of seed samples collected from a given population in 1959 and 1960 varied significantly. The significant variations from population to population are thus at least partially genetic in origin.

5. In preliminary experiments, seed neutron sensitivity of four species was the inverse of gamma sensitivity. The RBE of neutrons was higher for the species more resistant to gamma rays than more gamma sensitive species.

6. Dormant and active seed of four species were exposed to sixteen combinations of fast neutrons and gammas. Significant neutron-gamma interactions indicate the effects of these two types of radiation are not additive in either dormant or physiologically active seed.

7. When soaked and non-soaked seed samples of four species were exposed to 16 combinations of fast neutrons and gammas, water content, rate of physiological activity

and interactions of these variables modified gamma and fast neutrons effects differently. This is further evidence that these two types of radiation have different modes of action.

8. Soaked and non-soaked seed of five species were exposed to four levels of gamma rays and other samples of the same species to four levels of fast neutrons. Increased water content reduced gamma sensitivity and increased neutron sensitivity of dormant seed. This substantiates the inference that fast nuetrons and gammas have different modes of action. Increased water content of one species with physiologically active seed increased both fast neutron and gamma sensitivity.

9. The effects of water on gamma and fast neutron sensitivity of *Fraxinus americana* L. seed are not short term effects but presist during three years of stratification or three years of dry storage at 0-5°C. Although the effectiveness of fast neutrons was reported independent of water content of barley seed (16), it is not in *Fraxinus americana*.

10. Tree seedlings of *Juglans nigra* L., *Fraxinus americana* L., and *Quercus alba* L. irradiated while dormant or physiologically active were planted in a secondary forest. The variations in survival and growth rates among these gamma irradiated species growing in a native habitat are evidence that an irradiated ecosystem inhabited by these three species would change as a result of differences in species radiosensitivity.

11. Trees grown from irradiated and non-irradiated seed are now bearing seed. Viability of seed of two species did not differ significantly from controls during a good growing season but did following an unfavorable season. Radiation could be a powerful population control mechanism and the full effects of seed irradiation can not be determined from short term experiments.

12. Three species of *Pinus* are now growing over nuclear waste disposal pits near Morehead, Kentucky as a biological check on the effectiveness of the waste disposal methods being employed.

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