X-Radiation of Southern Pine Seed at Various Moisture Contents

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The 789 references reviewed by PRAKKEN (1959) indicate the striking increase in the use of induced mutation as a plant-breeding procedure. For many horticultural crops having long reproductive cycles, BROCK (1957) considers the method more promising than conventional ones. With forest trees, induced mutation may likewise have an advantage over such time-consuming procedures as backcrossing. In sexually propagated plants, however, detriments to reproduction are a frequent results of mutation breeding. SMITH (1958) proposed: "Modification of mutagenicity of practical value would include ways of inducing beneficial mutation; with a minimum of such effects as chromosomal aberrations, physiological injury and sterility. Accumulating evidence indicates that it will be particularly profitable to ascertain ways by which factors in the organisrn and in the environment can be manipulated to modify radiation effects."

In the research reported here, southern pine seeds were x-rayed to learn how various rates, quantities, and qualities of rays, and their interactions with seed moisture content and pregermination treatment ("stratification"), affected germination and seedling growth. Possible evidence of radiostimulation was also sought. The species were loblolly pine (Pinus *taeda* L.), slash pine *(Pinus elliottii* ENGELM. var. *elliottii),* longleaf pine *(Pinus palustris* MILL.), and shortleaf pine (Pinus *echinata* MILL.).

Because irradiation breeding with conifers is relatively new, the authors discuss some as yet unconfirmed suggestions and review literature dealing with the subject.

Literature

X-rays Compared to Other Rays

With x-rays more than with other types of irradiation, modification of the rays will influence the rate and type of mutation (EHRENBERG and NYBOM, 1954). CALDECOTT et *al.* (1957) report that x-rays approached neutrons in ability to produce mutations from seed with high water content. With high voltages and appropriate filters, they were similar to gamma rays (KONZAK, 1957). They are also likely to cause more physiologic damage and to be less controllable than other types of irradiation (KONZAK, 1956).

Data on the sensitivity of conifer seed to different kinds of rays are few and in some instances conflicting. GUSTAFSSON and SIMAK (1958) irradiated Scotch pine seed with x-rays (150 KV, 10 MA, focus 50 cm, filter 0.5 mm Cu + 1.0 mm Al, 20 r/min) and gamma rays at the same rate. LD-50 (the dosage at which germinability is reduced by 50 percent relative to the control) for x-rays was 1500 r; for gamma rays 2300 r. LD-50 for x-radiated spruce seed was 3000 r. R. E. SCHOENIKE (personal communication) recovered very few loblolly seedlings from 2000 r or more of gamma rays, but MAY and POSEY (1958) noted slight damage to germinability of unstratified slash pine seed with 5000 r of gamma rays delivered at 5417 r/min.

MÜLLER-OLSEN and SIMAK (1954) and MÜLLER-OLSEN et *al.* (1956) found that fresh seed of Scotch pine and Norway spruce had an LD-50 at about 900 r of x-radiation (12 KV, 25 MA, focus 25 cm). SIMAK and GUSTAFSSON (1953) had similar results for Scotch pine but only about 25 percent germination of Norway spruce for that dosage (175 KV, 15 MA). HEIMBURGER (1960) obtained an LD-50 at 2000 r with x-radiated white pine seed. A. D. RHODES (personal communication) reported germination of white pine seed x-radiated at 500 r as good, at 2000 r as fair, and at 8000 r as almost nil; with thermal neutrons, germination was low after 1 hour of exposure and nil after 2 hours.

HAGBERG et *al.* (1958) review and present new evidence that sparsely ionizing radiations like x-rays do produce mutagenic effects different from densely ionizing radiations like neutrons. However, both BROCK (1957) and KONZAK (1957) maintain that the relative efficiency of the different ionizing radiations for beneficial mutants is not known and that there is little justification for using one kind in preference to another except as is expedient.

Influence of Factors in the Organism

Physiologic state of the cells has more influence on response to irradiation with x-rays than with other rays. According to KONZAK (1957) sensitivity of barley seed to x-rays may vary 20-fold with moisture content. EHRENBERG and NYBOM (1954) found that all but very short pre-soaking of barley seed increased x-ray sensitivity many times. KAMRA et. *al.* (1960) have evidence that the sensitivity of barley seed after soaking is due not only to indirect effects of water but also to loss of energy-rich substances through leaching. SATO and YOSHIO (1951) noticed that soaking Japanese red pine seed increased sensitivity to fast neutrons.

When EHRENBERG and NYBOM (1954) allowed barley seed to come into equilibrium with air at various relative humidities up to 100 percent, sensitivity decreased with increasing moisture content. SHAPIRO (1956) emphasized that sensitivity according to moisture content varied with species.

KONZAK (1957) summarized his research on the most efficient moisture content: "The frequency of mutations is greater and the spectrum different when moist rather than dry seeds are exposed to the same dose of x-rays. However, the *highest total* mutation frequency has been obtained from the application of high radiation doses to seeds in their more radio-resistant dormant condition." BROCK (1957) cautioned that it was not yet possible to define the environmental conditions producing the greatest yield of beneficial mutants.

Radiostimulation

SAX (1955) found no valid experimental evidence for radiostimulation; he noted that most of the results were not statistically evaluated and might be attributed to chance. BEATTY and BEATTY (1956) discovered that an x-ray dose that stimulated at 5 r/min was inhibiting at 15-50 r/min. Their data indicated "... that there might be a minimum dose or method of administration where no effect would be observable and an optimum intensity where the greatest stimulation would be observed. Increased radiation beyond

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the optimum probably would produce physiological changes detrimental to the growth of the plant."

For soaked or unsoaked Japanese red pine seed, exposure to fast neutrons at certain doses increased germination, survival, and growth (SATO and NISHINA, 1951). TRALAU (1957) reported stimulation in germination rate and growth from spruce seed gamma-radiated mostly at 150-300 r. Increased germination or growth from x-radiated conifer seed has been reported at 300-400 r (MÜLLER-OLSEN and SIMAK, 1954; MÜLLER-OLSEN *et al.*, 1956; and SIMAK and GUSTAFSSON, 1953). For four species of pines, including loblolly, F. I. RIGHTER (personal communication) noted some stimulation of germination and seedling height growth following low dosages of x-rays. R. E. SCHOENIKE (personal communication) noted the same for loblolly pine receiving 500 r of gamma rays. VIDAKOVIĆ (1960) noted increased "germination power" of pine and spruce seed from gamma irradiation at 748 r or lower.

Neither GUSTAFSSON and SIMAK (1958) nor BALDWIN (1936) could detect stimulation from x-radiation of conifer seed.

Present Investigations

Most of the following data are from the third of three experiments, but two others are reported briefly to summarize all results from x-radiation at the Southern Forest Experiment Station.

Experiment I

The goals of the first experiment were early and profuse fruiting. Seeds x-rayed by the Maria Moors Cabot Foundation for Botanical Research, Harvard University, were sent to the Southern Forest Experiment Station by Dr. SCOTT PAULEY. They consisted of longleaf pine at 2000 r, slash pine at 2000 r, and loblolly and shortleaf pine at 1000 and 4000 r, plus untreated controls. After 1 year in the nursery, living trees per 100 seeds sown were 13 for loblolly at 1000 r, and 10 for shortleaf at 1000 r. Dosages of 2000 and 4000 r were lethal for both species — no seed germinated. In untreated controls, 50 trees resulted per 100 seeds sown.

The 136 survivors, about equally divided as to species and treatments, were outplanted in south Mississippi in 1952, with seedlings from treated and untreated seed in alternate rows. Heights 2 years after outplanting were:

	Control	Irradiated (1000 r)
	Feet	*Feet*
Loblolly pine	5.3	4.4
Shortleaf pine	3.9	3.3

Survivals after 5 years are shown below. The probability for chi-square when data of the two species are pooled is .10 — .25.

	Control	Irradiated (1000 r)
	Percent	*Percent*
Loblolly pine	93	86
Shortleaf pine	82	74

By 1959 three loblolly control trees and two irradiated trees had flowered. Cone bearing for shortleaf trees 7 years after outplanting was:

	Control	Irradiated (1000 r)
	Number of trees	*Number of trees*
With female cones	15	12
Without female cones	14	19

Average number of cones per bearing tree was 17 for the control, 10 for the irradiated. Thus survivals, heights, and flowering have been less for the irradiated material than for the controls.

Experiment II

Armed with an idea of sensitivity from results of the prior experiment, R. E. SCHOENIKE and P. C. WAKELEY of the Southern Forest Experiment Station initiated a test to determine how the production of mutations is affected by dosage and seed conditions.

J. U. HIDALGO irradiated loblolly seed at 9 r per minute to secure the dosages listed in *table 1*. In 1954, unstratified seeds were sown at Crossett, Arkansas, with 500 seeds per treatment in unreplicated plots, and outplanted in 10-tree plots with 3 replications. In 1955 irradiations were with both pre-stratified and dry seeds that were subsequently sown at Crossett, in southern Arkansas, and at the Southern Institute of Forest Genetics, in southern Mississippi. Stratification was by cold, moist treatment for 23 days. Replicated outplantings consisted of 5- to 25-tree plots at both Crossett and the Institute.

Table 1. — Stand from x-radiated loblolly seed after 1 year in nursery.

Dosage	Crossett, Arkansas		Harrison County, Mississippi	
	Unstratified	Stratified	Unstratified	Stratified
r	*Percent*			
0	6	50	17	65
200	10	24	19	66
400	4	50	14	66
600	6	30	22	49
800	5	22	19	44
1000	4	26	22	38
Seeds per plot	200	200	100	100
Number of Blocks	1	1	2	2

For seed that had been stratified and then irradiated, the 1-year stand percentages, based on number of seeds sown, became poorer with higher dosages. LD-50 was about 1000 r. No effects were detected for germination percentages of dry seed, for nursery growth from dry or stratified seed, nor for survival and height up to 4 years after outplanting. Poorer survival from stratified and irradiated seed increased the growing space per plant in the nursery bed, and thus may have compensated for a reduced growth potential. With dry seed, irradiation effects in the nursery may have been obscured by the poor and irregular stand resulting from lack of stratification.

Experiment III

Experiment III was planned to answer the questions: What if seeds had been stratified after irradiation? Would growth differences have occurred in uniform stands? How reproducible are results?

The study was with slash seed from 1955 and longleaf and shortleaf from 1956. All seed was refrigerated until treated. Each species made up a 57-treatment, 5-replicate randomized block experiment. The number of seed assigned per plot varied between 50 and 200, i. e., amounts estimated to yield adequate stands despite stratification and radiation effects. Individual lots were packaged in cheesecloth squares formed into bags by twisting label wires around them.

In the early spring of 1957; the seeds were subjected to four moisture content (MC) treatments: (1) Continued dry storage at 5-10° C, (2) cold-moist prestratification in peat moss for 2 weeks, (3) prestratification for 3 weeks (ray rate and quality tests were restricted to this moisture treatment), (4) post-stratification for 3 weeks, (5) exposure to circulating air at 100% relative humidity (RH) for 1 week at 20° C, after which time seeds averaged 90% of their equilibrium MC's. *Table 2* shows the MC's as determined from oven-dry weights.

Table 2. — Moisture contents of seed prior to irradiation.

Species	Dry-stored seed	Exposed 1 week at 100 percent RH	Stratified 2 or 3 weeks
		Percent	
Longleaf pine	10.1	17.5	35.4
Slash pine	11.1	17.3	33.3
Shortleaf pine	8.3	13.4	30.5

Rays from a GE Maximar 250 set at 200 KVP and 15 MA, were varied as to dosage (200—1800 r), quality (filtration vs non-filtration), and rate (5, 25, and 125 r/min). Dosages of 200—1200 r were obtained up to 5 at a time by stopping a 5-sectioned rotor at specified times and putting on or off certain treatments. The 1800 r was obtained without rotation. Some of the seed were exposed to unfiltered rays (H. V. L. = 0.6 mm Cu) and others to rays filtered by 0.5 mm Cu (H. V. L. = 1.0 mm Cu). Rates, as calibrated by a Victoreen condenser-r meter 70, were reduced from 25 r/min to 5 r on the rotating disk by altering the target-to-sample distance from 50 to 112 cm. The 1800 dose was delivered at a rate of 125 r/min. without rotation.

A randomized block design was used with plots 2 feet long and 6 inches apart (i. e. half of single crosswise rows within the nursery bed). Each plot therefore had a square foot of growing space. Seeds were spaced by hand. Plots were subsequently thinned to 16 seedlings each and a slow-release nitrogen fertilizer was applied. After a year in the nursery, the seedlings were lifted and plot weights determined to the nearest 5 g.

Germination: — From lots of 250 seeds, germination percentages 1 week after emergence were, for the controls (non-irradiated, 3-week stratification), 80 for longleaf, 94 for slash, and 78 for shortleaf. At 1000 r or above seeds prestratified for 3 weeks had the least germination. Seeds poststratified had the most, and the other three treatments were intermediate *(fig. 1)*. Differences within a dosage were significant at the 1-percent level. The differences between pre- and post-stratification are similar to those found by Heaslip (1959) for some hardwood species.

At lower dosages some treatments were better than the controls. The most interesting case — though not statistically significant — is an 8-percent improvement for shortleaf seed prestratified 3 weeks and irradiated at 300 or 400 r.

Filtering the x-rays with copper accentuated the sensitivity of all species *(fig. 2)*. The greatest change was at 1200 r, where germination percentages relative to the control were reduced from 62 to 40 for longleaf, 40 to 1 for slash, and 91 to 10 for shortleaf.

Reducing the rate from 25 to 5 r/min. for dosages of 200—500 r produced no statistically significant differences. Perhaps some trend was indicated in shortleaf, where germination was greater at 200, 300, and 500 r when applied at 5 rather than 25 r/min.

Green Weight: — There were no significant differences in weight per longleaf plant with various moisture and unfiltered ray treatments.

Stratifying slash pine seed predisposed it to irradiation damage. The mean weight of 28.3 g for the stratified series is highly significantly less than the 31.4 g for seed not prestratified.

Table 3. — Green weight per shortleaf plant after 1 year.

Dosage	A Dry	B Irradiated after 1 week at 100% R.H. + irradiated before 3 weeks of stratificat.	C Irradiated after 3 weeks of stratification + irradiated after 2 weeks of stratification
r	*Grams*	*Grams*	*Grams*
200	17.3	20.1	21.3
300	21.2	21.3	22.3
400	20.1	21.7	22.5
500	18.8	19.3	19.1
600	18.6	21.2	22.0
800	19.2	21.1	18.9
1000	19.3	20.2	19.7
1200	19.0	20.9	17 9
1800	19.1	19.2	16.3
0 (control)	22.4	—	21.2

Shortleaf response *(table 3)* fell into three categories: *A* — Plants from dry seed made highly significantly poorer average growth than those from the other two moisture treatments. The effects of various dosages on dry seed did not differ significantly. *B* — The average growths of the 100-percent RH series and the post-stratified series were similar and these two series have been pooled as the B column. The dosage treatments are not significantly different. *C* — Seed prestratified did not differ significantly in mean growth from B, but its dosage-mortality curve had a significantly different slope, i. e., there was increased damage at high doses and stimulation at low. The stimulation is evidenced by the relative weights of the seedlings: 22.4 g for the 20 plots receiving 300 and 400 r vs. 20.6 g for the 15 plots receiving 0 and 200 r. The difference of 8% is significant.

Filtering increased growth damage to all species *(fig. 3)*. For the average of the 1200 and 1800 r dosages, decreases in mean growth from filtering were 12% for longleaf, 28% for shortleaf, and 49% for slash. Growth was better at 1800 r than at 1200 r. It is not known whether this result is similar to one noted by Schwartz (1954) with maize or related to the 125 vs. 25 r/min delivery rate.

Changing the rate from 25 to 5 r/min decreased weights for most dosages and all three species, but the differences were not statistically significant. However, it is of interest that, for shortleaf at the 300, 400, and 500 r dosages, growth responses were the opposite of germination response: where one was higher, the other was lower.

Discussion

Radiostimulation is in disrepute for most crops. May it have value in forestry? How long must the tree breeder wait to detect mutations? What is the effect of seed moisture on radiosensitivity? What treatments can be recommended for producing mutations? These questions are discussed in the following paragraphs.

Radiostimulation of many crops, including forest trees, has been observed, though usually not substantiated statistically. While possibly a 1 in 20 accident of sampling, the increase in weight of shortleaf seedlings in this study is encouraging. That the greatest stimulation occurred in

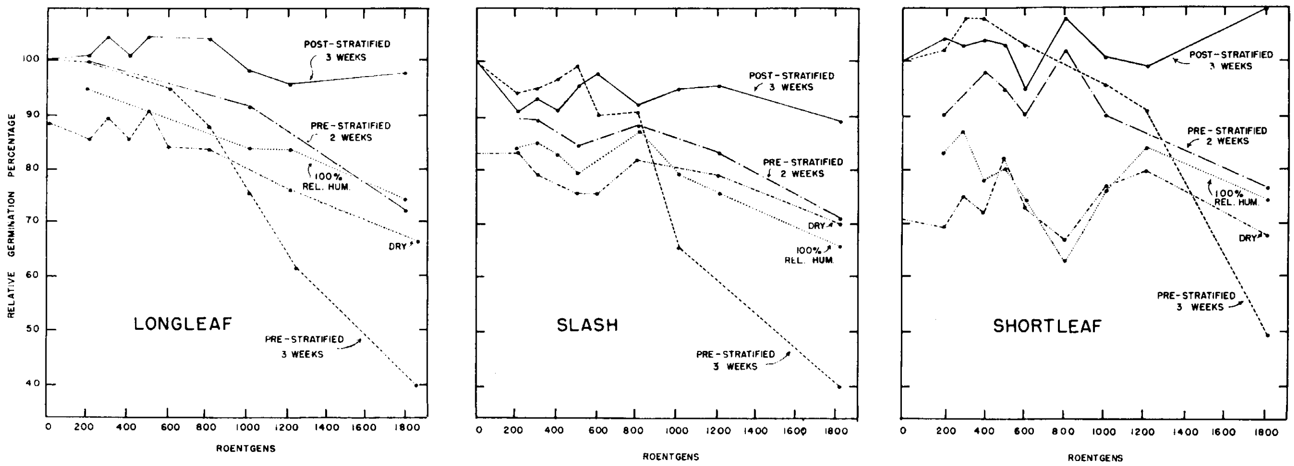

Figure 1. — **Germination percentages (relative to stratified but non-irradiated control) at various dosages of x-rays.**

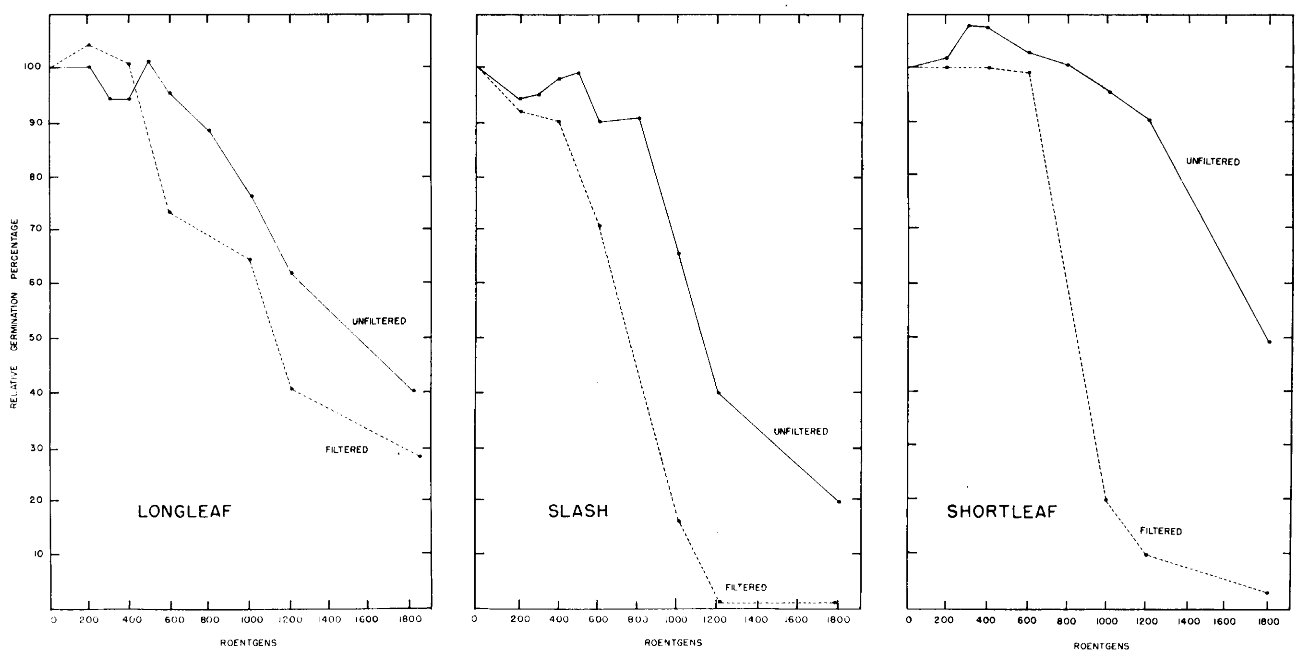

Figure 2. — **Germination percentages (relative to stratified but non-irradiated control) of seed receiving various dosages of filtered and unfiltered x-rays.**

shortleaf, the species most likely to require stratification, harmonizes with the suggestion of Ehrenberg (1955) that such effects are most likely to occur where there is an inhibiting seed condition.

How long must the tree breeder wait to detect mutations and what types have been reported in the first generation (M_1)? Theoretically, dominant mutations should be visible in the M_1. Gustafsson *et al.* (1950) have demonstrated growth heterosis under certain environments resulting from heterozygosity where one of the pigment alleles is a lethal. The irradiation of pollen to obtain such heterozygosity is recommended for forest trees by Osborne (1957). Abnormal M_1 characters in conifers are listed by Gustafsson and Simak (1958) as a mosaic of light spots in normal green leaves and a solid abnormal dark green; and by Toyama (1954) as linear cotyledons, stem bending, and chlorophyll aberrations. Some of these may prove to be mutants. However, current practice with sexually propagated crops is to select in the M_2 generation, where recessive mutations are also apparent and where many detriments to reproduction have been eliminated.

Our MC results complement those of Gustafsson and Simak (1958). They found spruce seed to be sensitive at MC's of 3%, while our studies indicated severe irradiation damage at 33%. Unless the differences are attributable to genera, it would appear that both high and low MC's can predispose sensitivity.

How can these MC results be used to produce mutants? Although further research is badly needed, the writers tentatively recommend that pine seed be irradiated when radio-resistant, i. e. at a moisture content intermediate between the extremes mentioned above. This conforms to recommendations for other crops. Stratification, if needed, can follow irradiation.

How high should the dosage be? Probably enough so that slight physiologic effects can be detected. The present data do not indicate the precise dosage. For physiologic predictions, Shapiro (1956) prefers growth damage to dosage-mortality.

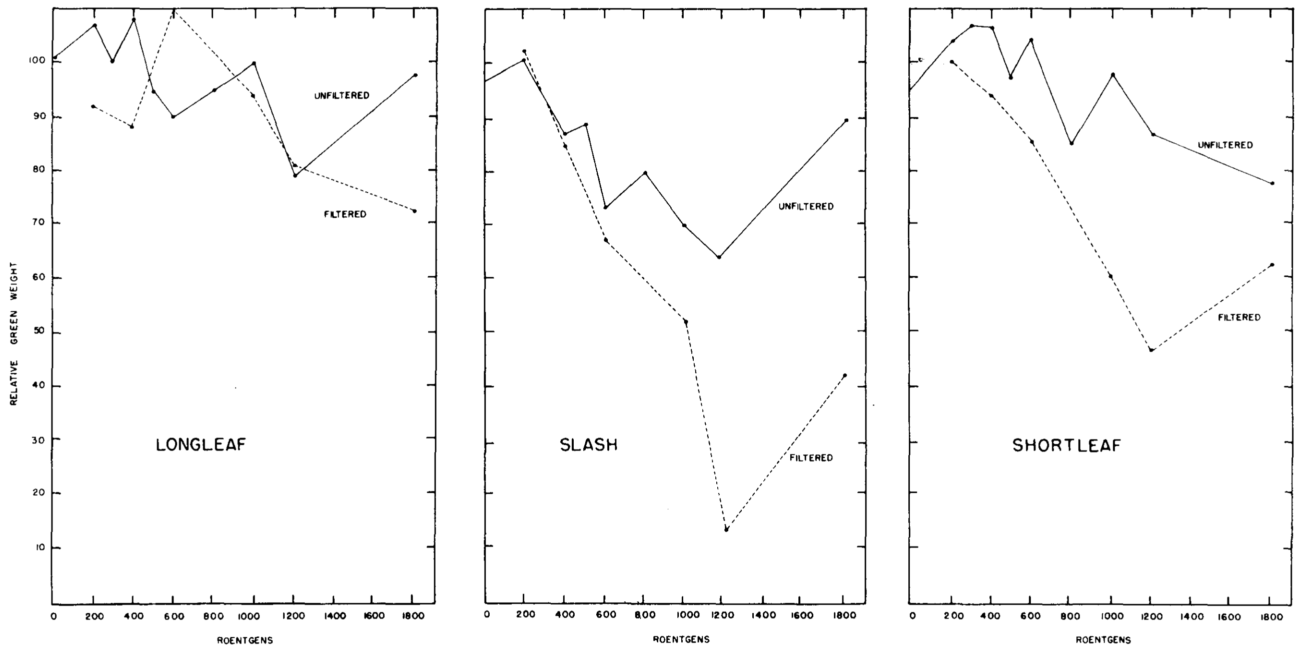

Figure 3. — Green weight (relative to mean of 0 + 200 r treatments of seedlings) from seed x-radiated with filtered and unfiltered rays.

Summary

Loblolly and shortleaf pine seeds receiving 1000 r of x-rays were capable of less survival and growth than untreated seed, and cone production was not stimulated during 2 to 7 years after outplanting. A dosage of 2000 r was lethal to longleaf and slash pine; this dosage was not tried on loblolly and shortleaf pine but 4000 r was lethal to these species.

In a second experiment, a dosage-mortality response in 1-year survivals was obtained when stratified loblolly seeds were irradiated with 200, 400, 600, 800, and 1000 r of x-rays. LD-50 was approximately 1000 r. Irradiation did not detectably affect the germination of dry seed, nursery growth of seedlings from dry or stratified seed, or (up to 4 years after outplanting) the survival or height-growth of trees from dry or stratified seed.

In a third experiment, seeds of longleaf, slash and shortleaf pine were subjected to various moisture and ray treatments. Prestratification for 3 weeks in conjunction with dosages of 800 r and above caused significant to highly significant reductions in germination percentages. LD-50 averaged 1600 r for the three species. Seed prestratified for only 2 weeks had the same MC as the 3-week seed, but was intermediate in sensitivity. Germination was also intermediate for dry seed and seed exposed to 100% RH, while high germination was obtained from seed irradiated dry and then stratified.

Changing the rate from 25 to 5 r/min resulted in no significant differences.

Filtering the rays by copper substantially decreased germination and green weight when seed had been pre-stratified 3 weeks, except for the weight of longleaf.

Some species characteristics were that: (1) Stratifying slash pine seed predisposed it to growth damage from high doses of irradiation. The reduction was highly significant for both the 2- and 3-week stratification period but more pronounced for the latter. (2) Likewise, pre-stratification of shortleaf caused similar damage at high doses. With shortleaf seed exposed to 300 and 400 r, however, germination and green weight each increased 8% over the 0 and 200 r average. The latter difference is significant.

The following values, from pre-stratified seed and expressed as percentages of the control, exemplify relative sensitivity of the species to irradiation:

X-rays at 1200 r	Germination percentage			Green weight		
	Long-leaf	Slash	Short-leaf	Long-leaf	Slash	Short-leaf
Non-filtered	62	40	91	79	64	87
Filtered	40	1	10	81	13	47

Zusammenfassung

Titel der Arbeit: *Röntgenbestrahlung der Saat südlicher Kiefern bei verschiedenem Feuchtigkeitsgehalt.*

Versuchspflanzen von *Pinus taeda* und *P. echinata* aus Samen, die eine Strahlung von 1000 r erhalten hatten, überlebten in geringer Zahl und hatten nur ein geringeres Wachstum als solche aus unbehandelten Samen. Ihre Zapfenproduktion war dadurch 2 bis 7 Jahre nach dem Auspflanzen auch nicht stimuliert worden. Eine Dosierung von 2000 r war bei *P. palustris* und *P. elliottii* letal; bei *P. taeda* und *P. echinata* war sie nicht versucht worden. Dagegen waren bei diesen Arten 4000 r letal.

In einem zweiten Experiment konnte bei den einjährigen Überlebenden eine Dosierungs-Sterblichkeits-Beziehung erhalten werden, bei dem stratifizierte *P. taeda*-Saat mit 200, 400, 600, 800 und 1000 r bestrahlt worden war. Die Letal-Dosis von 50%, bezogen auf die Kontrollen, (LD-50) erhielt man bei etwa 1000 r. Die Bestrahlung wirkte nicht feststellbar auf die Keimung von trockener Saat, ebenso nicht auf das Baumschulwachstum der Sämlinge aus trokkener oder stratifizierter Saat, auch nicht auf das Überleben (bis zu 4 Jahre nach dem Auspflanzen) oder das Höhenwachstum der Bäume, die aus trockenen oder stratifizierten Samen hervorgegangen waren.

In einem dritten Versuch wurden Samen von *P. palustris, P. elliottii* und *P. echinata* bei verschiedener Feuchtigkeit und Strahlenbehandlung verwendet. Vorherige Stratifizierung von 3 Wochen in Verbindung mit Dosierungen von

800 r und mehr verursachten ein signifikantes bis hoch-signifikantes Absinken der Keimprozente. Die LD-50 lag im Durchschnitt bei den 3 Arten bei 1600 r. Samen, die nur 2 Wochen vor-stratifiziert worden waren, hatten zwar denselben Feuchtigkeitsgehalt (MC) wie die 3 Wochen stratifizierten Samen, doch verhielten sie sich in ihrer Empfindlichkeit intermediär. Die Keimung war auch intermediär bei trockener Saat und bei Samen, die einer relativen Feuchtigkeit (RH) von 100% ausgesetzt gewesen waren. Dagegen erhielt man eine hohe Keimfähigkeit bei bestrahlten trockenen Samen, die erst danach stratifiziert worden sind.

Veränderungen in den Dosierungsraten von 25 auf 5 r/min ergaben keine signifikanten Unterschiede.

Wenn die Strahlung durch Kupfer gefiltert wurde, nahm die Keimung und das Frischgewicht dann wesentlich ab, wenn die benutzte Saat 3 Wochen vor-stratifiziert worden war, mit Ausnahme des Frischgewichtes bei *P. palustris*.

Gefundene Art-Eigenschaften waren, wie folgt: (1) Die Saat von *P. elliottii* wird durch Stratifizieren so vordisponiert, daß hohe Strahlungsdosen Wachstumsschäden ergeben. Diese Wachstumsminderung war bei 2- und 3wöchigem Stratifizieren hoch-signifikant, und zwar bei letzterem ausgesprochener. (2) Bei *P. echinata* verursachte vorheriges Stratifizieren gleichfalls ähnliche Schäden bei hohen Dosierungen. Doch wurden hier die Keimung und das Frischgewicht um 8% über die Wirkung 0 und beim 200 r-Durchschnitt gesteigert, wenn nämlich die Samen mit 300 und 400 r bestrahlt worden waren. Dieser Unterschied ist sinifikant.

Folgende Werte sind Beispiele für die relative Empfindlichkeit der Arten gegenüber Bestrahlung von vorher stratifizierter Saat, ausgedrückt in % der Kontrollen:

Röntgenstrahlung 1200 r	Keimprozente			Frischgewichte		
	palustr.	*elliottii*	*echinata*	*palustr.*	*elliottii*	*echinata*
nicht gefiltert	62	40	91	79	64	87
gefiltert	40	1	10	81	13	47

Résumé

Titre de l'article: *Traitement aux rayons X des graines de pin du Sud-est des Etats-Unis à des teneurs en eau variables.*

Les graines de Loblolly Pine *(Pinus taeda)* et de Shortleaf Pine *(P. echinata)* qui ont reçu 1000 r de rayons X ont une survie et une croissance inférieures aux graines non traitées et la production des cônes n'est pas stimulée au cours des 2 à 7 années qui suivent la plantation. Une dose de 2000 r est léthale pour le Longleaf Pine *(P. palustris)* et Slash Pine *(P. elliottii)*. Cette dose n'a pas été essayée sur le Loblolly et le Shortleaf, mais une dose de 4000 r est léthale pour ces espèces.

Dans une deuxième expérience on a obtenu une relation entre la dose et la mortalité après un an de semis lorsque des graines de Loblolly Pine ont reçu, après stratificiation, des doses de 200, 400, 600, 800 et 1000 r. LD-50 correspondait approximativement à 1000 r. L'irradiation n'affecte pas de façon décelable la germination des graines sèches, la croissance en pépinière des semis provenant de graines sèches ou stratifiées et (jusqu'à ans après la plantation) la survie ou la hauteur des arbres issus de graines sèches ou stratifiées.

Dans une troisième expérience, des graines de Longleaf, Slash et Shortleaf Pine ont été semées, pour diverses teneurs en eau, à des doses variables de rayons X. La stratification préalable pendant 3 semaines, conjuguée avec des doses de 800 r et au dessus, a réduit de façon significative ou hautement significative les pourcentages de germination. LD-50 correspondait à environ 1600 r pour les 3 espèces. Les graines stratifiées pendant 2 semaines seulement avaient le même MC (teneur en eau) que celles stratifiées pendant 3 semaines, mais la sensibilité était intermédiaire. La germination était également intermédiaire pour les graines sèches et pour celles semées à 100% RH (humidité relative), alors qu'on a obtenu une forte germination pour les graines soumises d'abord aux rayons et stratifiées ensuite.

Le changement du taux d'irradiation de 25 à 5 r par minute n'amène aucune différence significative.

L'emploi de filtres cuivre diminue nettement la germination et le poids frais des semis lorsque les graines sont stratifiées pendant 3 semaines, sauf pour le poids frais des semis de Longleaf.

Les caractéristiques spécifiques sont les suivantes: (1) La stratification des graines de Slash Pine les prédispose à une réduction de croissance lorsqu'elles sont soumises à de fortes irradiations. La réduction de croissance est hautement significative pour la stratification pendant 2 et 3 semaines, mais plus prononcée pour le dernier traitment. (2) De même, la stratification préalable du Shortleaf Pine cause des dommages analogues aux fortes doses; cependant, la germination et le poids frais pour les graines exposées à 300 et 400 r sont augmentés de 8% par rapport à la moyenne de celles exposées à 0 et 200 r. Cette différence est significative.

Le tableau suivant où les graines pré-stratifiées sont exprimées en pourcentage du témoin montre la sensibilité relative des diverses espèces aux rayons:

Rayons X à 1.200 r	Pourcentage de germination			Poids frais		
	Longleaf	Slash	Shortleaf	Longleaf	Slash	Shortleaf
Non filtré	62	40	91	79	64	87
Filtré	40	1	10	81	13	47

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Needle Characteristics of Hybrid Pines

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The forest genetics program of the Institute of Forest Genetics,[2] Placerville, California, has produced many hybrid pines (genus *Pinus*), which are being tested in national forests, in various arboreta, and by cooperators elsewhere. Those hybrids which prove of value in silviculture will be planted extensively by foresters. Means of identification, recognition, and verification of hybrids, particularly young plants without cones, are needed. Pine leaves or needles, always present on living plants, have long been useful in identification. Their relatively constant characters generally are correlated with cones and other taxonomic characters. Likewise, needles provide vegetative characters for studying inheritance in hybrid pines at an early age.

The purpose of this article is to present in tabular form the results of studies of gross morphology and microscopic anatomy of leaves or needles of 42 pine hybrids (genus *Pinus)* made at the Institute of Forest Genetics since its establishment in 1925 and growing there by 1957. The tables may be useful in identification of hybrids and in studies of inheritance of characters. Formal botanical descriptions of these hybrids, including twigs, needles, cones, and other characters, and reports of performance will be published elsewhere.

Review of Literature

Gross characters of the needles of the various species in the genus *Pinus* are summarized in taxonomic references, such as SHAW (21), PILGER (16), and DALLIMORE and JACKSON (4). Several publications, some with keys, describe and illustrate the anatomy of needle cross sections; for example, COULTER and ROSE (2), SHAW (21), DOI and MORIKAWA (5), HARLOW (10), and SUTHERLAND (24). FERRÉ (8) made a detailed study of the juvenile forms of *Pinus* and related genera and summarized in tables the needle characters of 42 species. HELMERS (11) observed the variation in needle anatomy of *Pinus ponderosa* as related to environment and position on the tree.

A few recent investigations on needle anatomy of hybrid pines have appeared in Silvae Genetica after the studies reported here were made. MERGEN (14, 15) studied number of stomata per unit of needle length as a valid test to identify certain crosses. For the hybrid *Pinus peuce* × *strobus*, FOWLER and HEIMBURGER (9) analyzed stem and foliage characters including number of needle serrulations. VIDAKOVIĆ (25) and SCHÜTT and HATTEMER (20) investigated the needle anatomy of the hybrid *Pinus nigra* × *silvestris*.

Examples of the few studies of hybrid pine needles at the Institute of Forest Genetics may be mentioned. Needles were included in formal descriptions of two named hybrids, *Pinus* × *attenuradiata* STOCKWELL and RIGHTER (19) and *P.* × *murraybanksiana* RIGHTER and STOCKWELL (22).

STONE and DUFFIELD (23) confirmed by needle characters the hybrid origin of seedlings from difficult crosses made at Institute of Forest Genetics and germinated by embryo culture technique. Seedlings from the two crosses *P. lambertiana* × *armandii* and *P. lambertiana* × *koraiensis* were without stomata on the dorsal needle surface, as in both pollen parent species.

RIGHTER and DUFFIELD (18) observed that 2-year seedlings of *P. ponderosa* × *engelmannii* had longer needles than corresponding non-hybrid seedlings of the first parent. Foliage criteria were included in the study of natural hybrids between *P. coulteri* and *P. jeffreyi* by ZOBEL (27). CALLAHAM (1) found the properties of needle oils in hybrid pines to be intermediate or transgressive and not discriminative for studies of hybridization between the three species analyzed.

Materials and Methods

The Eddy Arboretum of the Institute of Forest Genetics contains one of the largest collections in the world of different kinds of pine trees *(Pinus)* — about 70 species and many varieties and hybrids (26). More than 25 artificial pine hybrids growing there have been listed in publications by the research staff (6, 7, 17). Thus, abundant material from living plants was available for study.

The two authors worked independently at the Institute of Forest Genetics, KENG in 1953 and LITTLE in 1956 and 1957. While preparing botanical descriptions of the hybrids, the junior author had an opportunity to verify on additional and older plants the observations of the senior author and to examine several newer hybrids not available earlier. The tables and text summarize the observation of both authors, while the illustrations were prepared by the first. Both authors are indebted to F. I. RIGHTER, J. W. DUF-

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