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## Effect of Recurrent X-Radiation on Germination and Seedling Vigor in Oats<sup>1</sup>

By R. ABRAMS and K. J. FREY<sup>2</sup>

The immediate effects of exposing oat seeds to high dosages of X-ray are generally twofold: (a) many of the seeds fail to germinate, and (b) the seedlings produced are stunted. It is generally accepted that these physiologic effects react the same as other environmental variation in biological materials. However, it is possible that even though the physiologic effects disappear in one generation that certain weaknesses may persist but remain hidden. Weakened linkages of some type within chromosomes could conceivably be a case in point. If this is the case, one might expect the effects to be cumulative with repeated x-ray treatment in successive generations, since the basic units affected are probably chromosomes and genes.

The study reported herein was conducted to determine to what extent the X-ray damage to oat seeds, measured as germination percentage and seedling vigor, was cumulative with recurrent radiation in one, two, and three seed generations. The measures of seedling vigor were plant height and weight per 100 seedlings.

### MATERIALS AND METHODS

The effects of recurrent radiation were studied on six oat varieties, Simcoe, Park, Bonham, Mo. 0-205, C.I. 6748<sup>3</sup>, and Clintland. The previous radiation history for each of the varieties was as follows:

Sample No.	1954	1955
1	25000r	25000r
2	None	25000r
3	None	None

All of the seeds used in these experiments were grown in the same field on the Agronomy Farm at Ames, Iowa, in 1955. Consequently, any differential sensitivity caused by the environment where the seeds were produced was at a minimum.

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<sup>3</sup>C.I. 6748 is a pure line selection from the cross; Santa Fe x Clinton<sup>4</sup>.

To obtain seed lots for this study composites were made consisting of identical numbers of seeds from each 1955 plant of a given variety and treatment. Since the number of plants surviving from radiated and nonradiated materials was not the same, larger numbers of seeds were taken from the radiated plants in making the composites. The total number of composites was 18, consisting of 6 varieties and 3 combinations of previous radiation.

Each composite was divided into two equal parts for tempering to different moisture levels. The two samples were adjusted to 14.9 and 20.9 per cent moisture by keeping the seeds for two weeks in desiccators over saturated sodium chloride solution and water, respectively. The seeds lots were then radiated with a G. E. Maxitron X-ray machine operated at 250 kvp and 30 ma, using a .25 mm. Cu+Al filter. The dose rate was 1305 r per minute and the distance from the anode to the center of the target was approximately 15.5 cm. The total dosage was 40,000r for the seed samples with 14.9 per cent moisture and 30,000r for those with 20.9 per cent. The seeds were radiated in a plastic container with 3 compartments; thus the 3 seed lots for one variety and moisture level were treated with the same dosage simultaneously.

Within 24 hours after the radiation treatment the oat seeds were planted in flats in the greenhouse. A plot consisted of 100 seeds sown one-half inch deep in a sterilized soil mixture of loam, sand, and peat in a ratio of 2:1:1. The experimental design was a randomized block with 3 replications and non-radiated seed, used as a check, was included 4 times in each replication. Each variety was planted in a separate experiment.

Germination percentages and plant heights, in centimeters, were determined for each plot 14 days after planting. On the same day the seedlings were cut at the soil surface, dried at 30 degrees centigrade, and weighed. The dry weights were adjusted to weight per 100 seedlings in grams.

The greenhouse study was repeated in the field in 1956 using the same varieties and moisture contents. However, the dosages were reduced to 20,000r and 30,000r for the high and low moisture levels, respectively. Each variety was sown in a separate experiment and the field design was a completely randomized block with the following entries; (a) each combination of moisture content and radiation dosage included 3 times (b) laboratory dried samples of each seed lot included 3 times (c) samples from each seed composite tempered over water and saturated sodium chloride solution included once each. A field plot consisted of 4 rows, 8 feet long. In each row 25 seeds were spaced approximately 4 inches apart in the row, making a total

of 100 seeds per plot. The plant survival percentages were determined 6 weeks after planting.

Since the relative responses of the oat varieties were similar only the averages of all varieties are presented herein. Each point on a graph or entry in a table is an average of 18 determinations.

#### EXPERIMENTAL RESULTS

The average germination percentages of the oat seeds from the four different X-ray dosages and moisture level combinations are presented in Figure 1. The two uppermost lines represent the grain with the moisture content of 14.9 per cent when grown in the greenhouse and field. The average germination percentage for seeds with this moisture content and X-ray treatment ranged from 60 to 80.5, while comparable values for seeds with 20.9 per cent moisture ranged from 15 to 40. Obviously, moisture content of the seed was a critical factor in sensitivity to X-ray treatment.

There was a tendency for lower germination percentages with an increasing number of successive generations of radiation. For the 40,000r treatment in the greenhouse and the two field treatments each successive generation of radiation resulted in a lower germination percentage. However, in the 30,000r greenhouse experiment the lowest average germination percentage was produced by the material treated only once. No explanation can be given for the dip in this curve.

The reduction in germination percentage with the X-ray treatment of successive generations of oat seeds may be due to two causes; (a) a carryover of effects from previous radiation treatments unconfounded with the 1956 radiation, and (b) the cumulative effects caused by repeated radiation. To determine whether either or both of these effects were operating the data presented in table 1 were calculated. The germination percentages of the  $X_2$  generations of the seeds treated in 0, 1, and 2 successive generations give a clue to the carryover effects of previous radiations. At the 14.9% moisture level the germination percentages range from 86 for seed lots which never received X-ray treatment to 83 with one and 82 with two previous radiations. With a seed moisture content of 20.9 per cent the results were similar but more extreme. The germination percentages were 72, 67, and 60 for the material with 0, 1, and 2 previous radiation generations, respectively. Without a doubt there was some carryover effect from previous generations which caused a lower germination percentage. This effect was accentuated by tempering the oat seeds to a higher moisture content. The data in the last

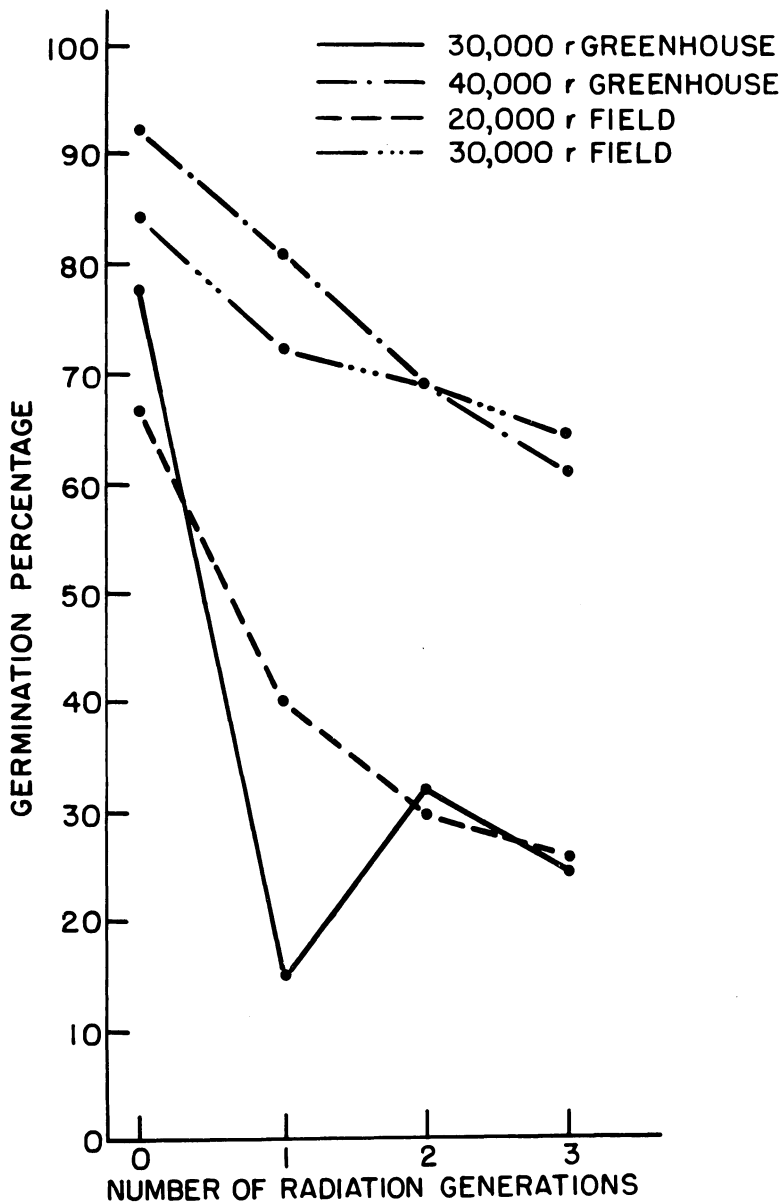


Figure 1. Germination percentages of  $X_1$  oat seeds treated with X-rays for none, 1, 2, and 3 successive generations.

column of table 1 show the germination percentages of the  $X_3$  generation of oat seeds treated once with X-ray. At the lower moisture content the germination percentage was as good as the untreated check while at the higher moisture percentage the reduction in germination noted in  $X_2$  persisted into the  $X_3$ .

**Table 1**

Germination Percentages of  $X_2$  Generation and the Relative Germinations of Oat Seeds Radiated for 0, 1, and 2 Successive Generations and Planted in the Field

Classification	Number of radiation generations			
	0	1	2	1*
Germination % of $X_2$ generation $X_1$ germination expressed as a percent of corresponding check**	14.9% moisture			
	86	83	82	87
Germination % of $X_2$ generation $X_1$ germination expressed as a percent of corresponding check	20.9% moisture			
	72	67	60	68
	56	46	42	51

\*Seeds in  $X_3$  generation.

\*\*Germination of the check ( $X_2$  for samples with previous radiation history) was considered as equal to 100 and the  $X_1$  germinations are expressed as a percent of this value.

If the effects of the 1956 X-ray treatment were unconfounded with previous radiation, the proportion of the seedlings surviving, expressed relative to the  $X_2$  germination percentage, should be similar for all seed lots. As shown in table 1 the relative per cent of  $X_1$  seedlings surviving the 1956 radiation decreased with an increasing number of previous radiations. The per cents of seedlings surviving, relative to the  $X_2$  generation, were 56, 46, and 42 at the high and 83, 83, and 78 at the low moisture levels for oat seeds irradiated in 0, 1, and 2 previous generations, respectively. Apparently the X-ray treatment effects were also cumulative over generations. A previous radiation had some weakening effect on the oat seeds that made them more sensitive to the 1956 X-ray treatment. The two effects suggested as (a) and (b) may be mutually exclusive events. This will be dismissed later in the paper.

The vigor of oat seedlings after differing numbers of generations of X-ray treatment were determined in two ways, seedling heights and weights per 100 seedlings. The data for these characters are shown in Figures 2 and 3, respectively. Since the trends in the data for both measurements of seedling vigor were so similar they will be discussed simultaneously. At the moisture content of 14.9 per cent and 40,000r there was a very marked reduction in seedling vigor from X-ray treatment. However, the reduction was about the same irrespective of previous radiation treatment. The seedling vigor points for 1, 2, and 3 successive generations of X-ray treatment virtually form a

straight line parallel to the abscissa of the graph. Apparently there was no cumulative effect of recurrent radiation when expressed as seedling vigor at the lower moisture content.

At the 20.9 per cent moisture and 30,000r treatment the seedling vigor tended to increase with an increasing number of successive generations of X-ray treatment. When interpreting this data it must be remembered that the seedling weights and plant heights were on the basis of surviving seedlings. This is obviously a case of fewer seedlings surviving with an increasing number of generations of radiation but the survivors were more vigorous. More light could be thrown on this point by studying the variability of seedling vigor in these samples.

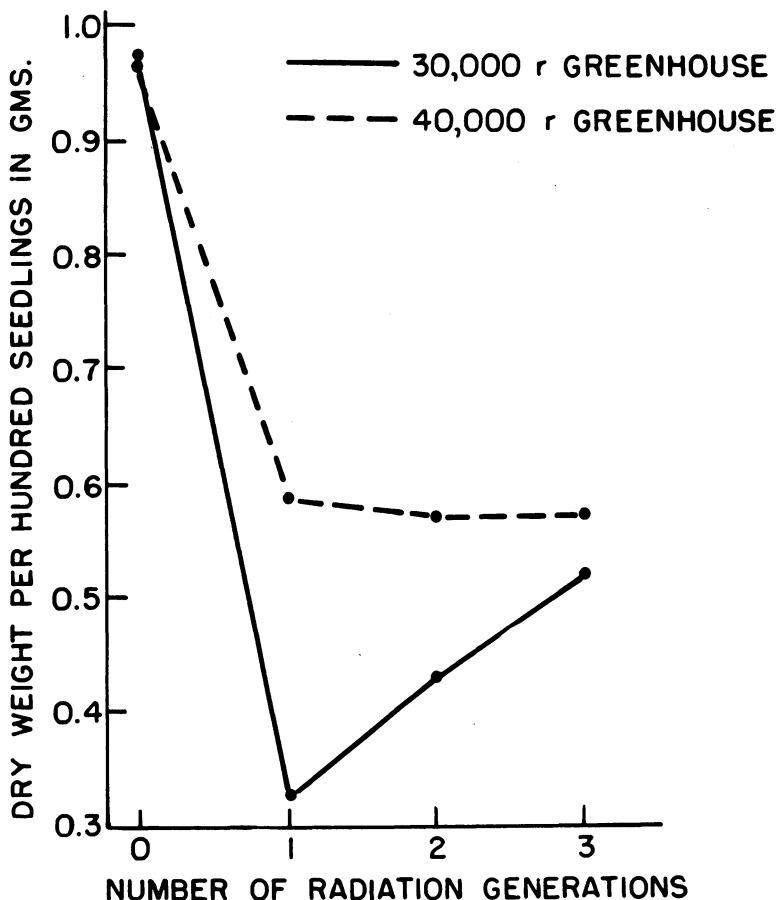


Figure 2. Dry weights per hundred  $X_1$  seedlings from oat seeds treated with X-rays for none, 1, 2, and 3 successive generations.

## DISCUSSION

If any of the oat varieties used in this study had possessed variability for genes which conditioned resistance to X-ray damage, it would be expected that each successive generation of recurrent radiation would have had less X-ray damage than the previous one. However, each succeeding generation appeared to become more sensitive when measured by germination percentage. However, this does

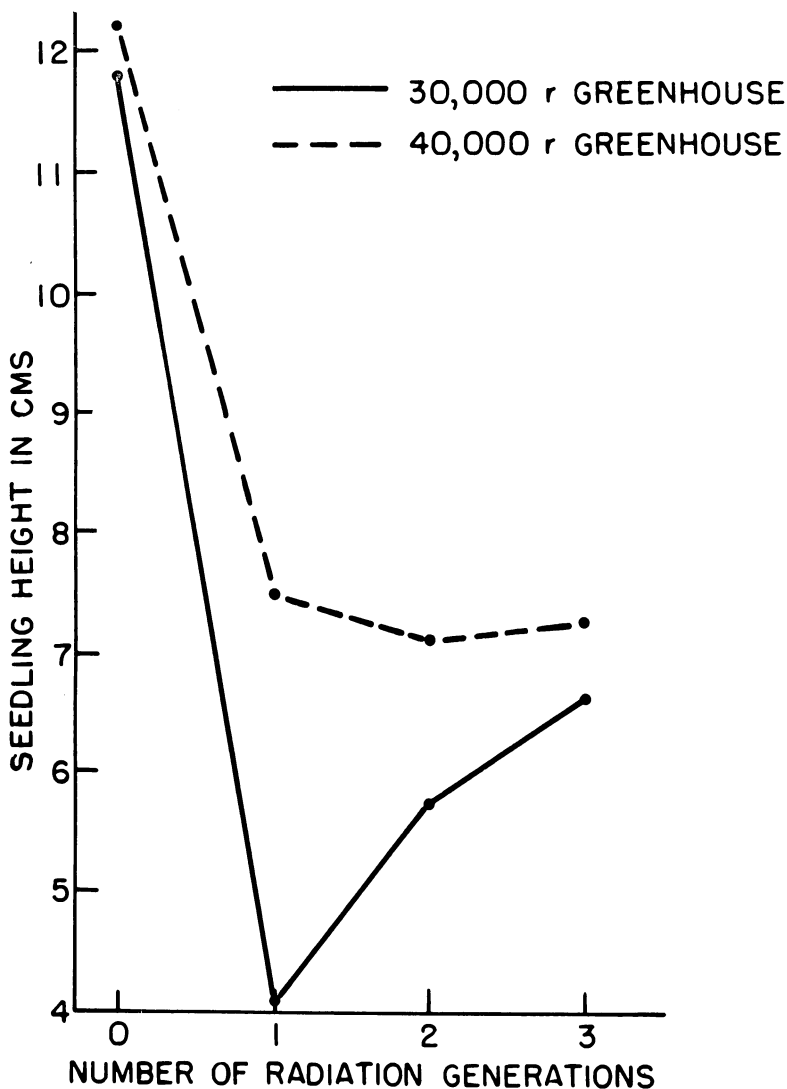


Figure 3. Average heights of  $X_1$  seedlings from oats seed treated with X-rays for none, 1, 2, and 3 successive generations.



not preclude the possibility that genes which condition resistance to X-ray damage do exist.

The greater lethality of oat seeds with an increasing number of successive generations of radiation could be due at least in part, to any one or all of the following causes: (a) a weakening, but short of actual breakage, of intrachromosomal linkage making the chromosomes more subject to breakage and which results in aberrations with repeated X-ray treatment. Presumably these aberrations would prove lethal to many seeds. (b) An accumulation of subnormal, but not quite lethal, mutations, one of which would not kill a seed, but two acting together could kill it. (c) Similarly it could be due to an accumulation of deleterious mutations or chromosome disturbances at homeologous locations in the different genomes. This would be possible only in a polyploid species like oats. (d) To a carryover effect in the endosperm or embryo cells which do not contribute to the heredity of the next generation.

The case for cause (d) can be eliminated rather quickly. Since each growth cycle had to pass through a seed generation before being re-radiated it is unlikely that such an effect would be carried through the gametes. Plasmagenes might be affected but these could probably be classed as hereditary units.

Since the effect of radiation was measured in the  $X_1$  generation any mutation which killed the seeds would have to be either a dominant lethal or a homozygous recessive lethal. Experience has shown that the frequency of dominant mutations is extremely small. Furthermore, the frequency of recessive mutations occurring in the two genes at a given locus is the product of their mutation rates, and thus very remote. For these reasons the reduction in germination percentage in the  $X_1$  with increasing generations of recurrent radiation can probably not be explained on the basis of (b).

This does not negate the fact that deleterious mutations do occur and to a certain extent accumulate with recurrent radiation. Evidence for this is found in the relationship between germination percentage in  $X_2$  populations and the number of successive generations of radiation. The seed with no radiation germinated highest and those with one and two radiated generations germinated intermediate and lowest, respectively. Probably a good share of this reaction was due to the uncovering of recessive lethal mutations upon selfing.

The effects of recurrent radiation upon oat seeds seems to be most easily explained by chromosomal disturbances. The differential effect of X-ray treatment at the two moisture levels can be most easily explained on the basis of chromosomal disturbances. The regression

line of reduction in germination (figure 1) was much steeper at 20.9 per cent moisture than at 14.9 per cent even though the X-ray dosage was lower in the first. It is quite commonly accepted that a higher moisture content causes a greater rate of metabolism which in turn is associated with greater chromosomal damage by X-rays. The seedling vigor of X-rayed samples with 14.9 per cent moisture was nearly the same irrespective of the number of generations of radiation while that for the 20.9 per cent sample increased. Since these are measurements on surviving seedlings it would lend evidence that seeds with a weakened chromosomal constitution were increasing and were killed at the 30,000r and 20.9 per cent moisture level more readily than at the other treatment.

Under cause (a) weakened chromosomal linkages would result from radiation effects which were not great enough to cause permanent fragmentation of the chromosomes, but weakened internal linkages, so that the chromosomes were more subject to fragmentation. With recurrent radiation the number of points where this could occur would increase. Supposedly, the chromosomal aberrations caused in this manner would be lethal.

The other chromosomal disturbance which could account for increased lethality of the oats with a greater number of recurrent radiation cycles would be explained by (c). The deletion of a chromosome segment in one genome of hexaploid oats might not be lethal because the homeologous segments in the other genomes would be present. Essentially such a strain would be equivalent to a nullisomic for a chromosome segment. If the segment in question were especially deletion labile, repeated radiation may delete the homeologous segment in another genome which probably would cause lethality.

These two hypotheses can be tested by comparing the effects of recurrent radiation on diploid and hexaploid oats. If cause (a) is responsible both types should become more sensitive with an increasing number of recurrent radiation cycles. However, with cause (c) the diploid would not show this phenomenon while the hexaploid would. Such experiments are now being conducted.

#### SUMMARY

Oat seeds which had been radiated in 0, 1, and 2 successive generations were tempered to moisture contents of 14.9 and 20.9 per cent and treated with 40,000 and 30,000r of X-ray. In greenhouse and field tests the germination percentages decreased with increasing numbers of recurrent radiation generations. The vigor of surviving

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seedlings was greater with increasing recurrent radiation at the high moisture level but not at the low one.

It was postulated that many deleterious mutations must have accumulated. However, the increasing effects of radiation measured in the  $X_1$  generation were probably due to an increase in the chromosomal aberrations.

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