STIMULATIVE EFFECTS OF X-RAYS ON PLANT GROWTH[1]

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(WITH FOUR FIGURES)

Introduction

During the period since the discovery of x-rays by RÖNTGEN in 1895, a vast amount of work has been done in which these radiations have been used for clinical diagnosis and therapy. The practical applications of x-rays in medicine and surgery make it necessary to know the effect which x-rays produce upon the living organism. Many investigators have suggested on the basis of general observations that small doses of x-rays may stimulate cellular activity and growth, but convincing proof of such action has been wanting. In more recent years such claims have been discounted in favor of the belief that x-rays are always more or less destructive in action, and tend to retard growth.

It is not the purpose of this preliminary report to survey the literature dealing with the effects of x-ray treatments upon plants. It has been found that every part of the plant body can be profoundly modified by appropriate treatments. Cytological and histological examination of treated cells and tissues reveals striking changes in the organization of the protoplasm and of organs derived from the treated meristems. Most frequently the results described are of a destructive nature. The protoplasm is partially disorganized; chromosomes are vacuolated or fragmented; the cell division mechanism functions imperfectly, showing unequal distribution of chromosomes, non-disjunctions, translocation of pieces of chromosomes from one to some other non-homologous chromosome, etc. Gene changes may be produced, often injurious in character, with resulting lethal effects and tendency to sterility. The results obtained by MCKAY and GOODSPEED (**5**) on cotton are typical. Many mutations have been induced in maize and barley (**7, 8**), and tobacco (**1**), but it has been questioned whether there are any progressive evolutionary changes induced by x-ray treatments.

All vegetative parts are subject to injury by x-rays. Root tips may become bulbous and swollen, with tumor-like enlargements in which giant cells may occur. Stems become fasciated under strong treatments. Leaves are injured readily; they become asymmetric and crumpled in appearance, develop deep sinuosities, and often show irregular development of chlorophyll. The sunflower shows these injuries in typical fashion, the leaves becoming pocked and marked as though they were suffering from a mosaic

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disease. Even the flowers of plants rayed in seed or seedling stages may show fasciation or various teratological modifications. Some of these have been described for the sunflower and tomato by JOHNSON (**2**, **3**).

On the other hand, one can find a dozen or more claims in the literature that x-rays in small doses are stimulative. In some cases increased yields have been claimed for crops grown from x-rayed material. Such claims have been reinvestigated in some cases, and the stimulative effects denied. JOHNSON (**4**), for instance, has not been able to substantiate such claims made for the potato. However, some increase of yield has been reported for x-rayed potatoes at the New Jersey Agricultural Experiment Station. PATTERSON and MULLER (**6**) have found that induced point mutations in *Drosophila* (presumably caused by chemical changes in the genes) may cause increased vigor in some cases. They argue in favor of the possibility of progressive x-ray mutations with endless eventual potentialities.

As a result of our experiences with the use of x-rays on plants it is believed that stimulative effects may be consistently obtained if appropriate conditions are employed. Possibly these stimulative phenomena have not been regularly detected in the past because the intensity of the radiations have been too great, or possibly because the x-ray beam contained too large a proportion of long wave-length radiation. Deleterious effects are consistently obtained in our work when unfiltered radiations are used, and we believe that these harmful effects mask the stimulation that occurs when the beam is properly filtered. Filtration of the radiation, of course, affects the wave-length constitution of an x-ray beam profoundly. It not only reduces the intensity of each wave length throughout the x-ray spectrum, but also changes the relative proportion of the energy supplied by each wave length throughout the spectrum. The shorter radiations suffer much less absorption than the longer radiations; and for practical purposes the longest x-rays are so strongly absorbed by aluminum or copper filters that filtration through such metal plates practically removes them from the beam.

Since filtration affects both the intensity and relative composition of the beam, and since we have not yet differentiated these effects in our work, we are not in position to discuss the nature of the x-ray action. Until further experiments are done we cannot say whether the stimulating effects that are obtained when the beam is filtered are due to the fact that harmful long wave-length rays are removed, or whether they simply indicate that stimulation follows low intensity irradiation, regardless of wave length, and is masked by injury if the intensity is greater, regardless of wave length.

Believing that the dosages in common use for treatment of plants were much too large, we have used very small doses. The intensity of the radiations used is expressed in Röntgen units measured with a Wulf ionometer,[2] the measurements being taken in air without the effect of back-scattering of the beam by solid material. We are indebted to DR. PAUL C. HODGES, Röntgenologist of the University of Chicago, for the calibration of our instrument, and for many helpful suggestions.

In these preliminary experiments we are using about 100 pk. KV., 5 ma., 1-mm. aluminum screen. Under these conditions the instrument delivers about 38 r-units per minute at a point 30 cm. from the target, the distance used in these experiments. Our experimental material is exposed on cellucotton pads in glass dishes resting on a lead-covered table. It undoubtedly received slightly higher doses than were computed in air because of a slight amount of back-scattering of the radiations. But the computation of the dose in air is a standard method of measuring the dosage. In some instances our best results have been obtained with 1 minute or less, a total of 30–40 r-units. In most cases maximum stimulation has been obtained with not more than 2 or 3 minutes; and with 4 or 5 minutes the effect is already one of retardation of growth.

It is evident at once that investigators who have been using from one to ten erythema doses as light doses, are using extremely heavy doses. The erythema dose is a rather rough unit of measurement, and may be defined as that dose of x-rays that just fails to produce a detectable change in the normal human skin. It is at best a vague designation, but is still much used. It seems much better to adopt the more accurate r-unit. It is generally accepted that the physical equivalent of the erythema dose is approximately 600 r. The Holzknecht is also used in expressing x-ray doses, and this is approximately 120 r.

The optimum dosage for different kinds of plants is probably specific, and must be determined by experiment for each species and varietal strain. A number of common plants seem to respond best to dosages between 30 and 120 r.

Methods

In order to make it possible to repeat our procedure, the details of preparation of the seeds for treatment are given. Seeds of such plants as corn, wheat, oats, and sunflower have been used. They are placed for 24 hours in a moist chamber upon a layer of cellucotton saturated with distilled water, and kept at a temperature of about 22° C. The seeds are used without sterilization, and lie in contact with the wet substrate on one side, and in contact with moist atmosphere on the other side. They are not

[2] Small-chamber instruments of this sort are intended primarily for use with higher voltages and are somewhat inaccurate at lower voltages. Eventually the calibration will be checked with large-chamber instruments that are relatively insensitive to voltage change.

submerged during the period of preliminary imbibition and germination. At the end of 24 hours the seeds of all four species show incipient germination. The radicles protrude through the pericarps and enable one to know that the seeds are alive. At this stage the material for treatment and for controls is selected. Twenty or more seeds as nearly at the same stage of germination as possible (estimated by equal length of protruding radicles) are chosen and divided into two lots. One lot is left untreated, the other is placed upon fresh saturated cellucotton and treated at once for 1–5 minutes. Optimum effects are often obtained with 1, 2, or 3 minutes of treatment, according to species. Sunflower seems best at 3 minutes, corn possibly at 2 minutes, and some varieties of wheat at 2 minutes. In some cases wheat gives good results at 30 to 45 seconds or 1 minute. As soon as the raying is completed, controls and treated seeds are both planted in the same type of soil, or in sand culture, or on fresh saturated cellucotton in a moist chamber, depending upon the nature of the experiment. In the case of respiration experiments, controls and treated seeds are placed on a wet substrate in the respirometer immediately after treatment. During treatment the glass covers of the moist chambers or petri dishes are removed so that the only screen is the metallic aluminum screen. In the case of sunflower seeds the pericarps of the fruits are removed before treatment. They are also removed from the controls before planting. We have tried to avoid any differences except that of the treatment itself. Selection of seeds is practiced only to obtain material of uniform physiological activity for the controls and treatments.

Results

Wheat

The first tests with Marquis spring wheat indicated that it is sensitive to small doses of x-rays. The treated plants were decidedly more vigorous than the controls when the period of exposure was from 45 seconds to 1 or 2 minutes. By the time the plants were several weeks old (in soil culture), the treated individuals were taller and of ranker growth. The greatest difference was in the degree of tillering. The untreated plants showed 50 per cent. with one tiller each, while the treated plants showed 100 per cent. with two tillers each. Figure 1 shows the general appearance of the plants on September 17, after several weeks of growth.

Tests with Minhardi and Trumbull wheat gave us the impression at the time that the hardier variety (Minhardi) was less easily influenced by x-rays. The Minhardi wheat in the first tests seemed to show little stimulation, while Trumbull, a moderately hardy variety, showed plainly that its early development was hastened by treatment, but not so much as the Marquis spring wheat. At the present time we are not certain as to the order of these varieties with reference to degree of stimulation.[3] It is possible that varieties more stable toward cold treatments may also be more stable toward x-ray action. We believe the dosage is specific for each variety, and that a longer treatment may possibly be required by the hardier varieties to produce a given amount of stimulation.

CORN

The most interesting results were obtained with Madison Yellow Dent corn. It was noted that grains which had been treated emerged from the soil more rapidly. On September 22, seeds which had been imbibing water for 24 hours were treated 1–5 minutes, one series screened by aluminum, another treated without metallic screen. A third series, untreated, served as controls. Five days later the seeds treated through the screen showed 84 per cent. of emergence; the unscreened treated seeds showed 72 per cent.;

FIG. 1. Influence of x-rays on growth of wheat: Pot at left rayed 1 minute; at right, 45 seconds. Controls in middle pot. For other conditions see text.

and of the controls only 60 per cent. had emerged. Treated seeds kept in petri dishes always showed a more rapid elongation of coleoptiles than untreated seeds. We have removed such coleoptiles from the seeds at the end of three days and determined the fresh and dry weight of the coleoptiles. Treated seeds showed from 5 to 26 per cent. greater fresh weight than the controls, and from 3 to 16 per cent. greater dry weight. This suggests the possibility that there is a more rapid utilization of the endosperm reserves in seeds that have been treated.

When the treated corn seeds were grown for a few weeks, some very important differences were noted. Figure 2 shows corn grown from seeds treated 1–5 minutes under an aluminum screen. While the growth differences are visible, and somewhat irregular, the main differences in this set are not visible to the eye in the photograph. The plants treated for

[3] Work on these varieties of wheat is being continued by Miss BESSIE ZABELIN.

TABLE I

INFLUENCE OF X-RAYS ON GROWTH OF CORN

TREATMENT	STEM DIAM.		FRESH WT. ROOTS		DRY WT. ROOTS		FRESH WT. TOPS		DRY WT. TOPS		CHLOROPHYLL* FRESH WT.		CHLOROPHYLL* DRY WT.		
	mm.	%	*gm.*	%	*gm.*	%	*gm.*	%	*gm.*	%	%	*mg./cm.*^{2}	*mg.*	%	%
Control ...	5.63	100.0	54.7	100.0	5.11	100.0	49.7	100.0	4.655	100.0	0.1006	0.0125	5.03	0.874	100.0
1 min.	5.85	103.9	58.8	107.5	4.76	93.1	65.4	131.6	6.03	129.5	0.1341	0.0163	6.70	1.22	139.6
2 min.	6.73	119.5	54.7	100.0	4.79	93.7	86.1	173.2	7.67	164.7	0.1688	0.0219	8.44	1.43	163.6
3 min.	6.15	109.2	54.2	99.0	3.69	72.2	63.9	128.5	5.85	125.6	0.1517	0.0201	7.58	1.34	153.3
4 min.	6.47	114.9	60.6	110.8	4.80	93.9	86.6	174.2	7.30	156.8	0.1573	0.0205	7.86	1.57	179.6
5 min.	5.45	95.2	62.2	113.7	5.66	110.7	65.1	130.9	6.44	138.3	0.1407	0.0178	7.03	1.21	138.4

* Chlorophyll determinations according to GUTHRIE, made by Mr. G. B. ULVIN.

short periods (1–3 minutes) had thicker stems than the controls, or those treated 5 minutes. The treated plants looked and felt slightly more succulent, and were darker green in color. The fresh green weight of the tops was obviously greater in the treated plants than in the controls. Without detailed discussion we present in table I such differences as were measured. The chlorophyll differences need further investigation, as this darker green color was not noticed in the oats, wheat, and sunflowers.

The irregular growth of the 3-minute plants in figure 2 may have been caused by a defect in the instrument which was not discovered and corrected until after several lots of seeds had been treated. In table I the most important data are those on dry weight increase (column 11) and those on chlorophyll increase (column 16).

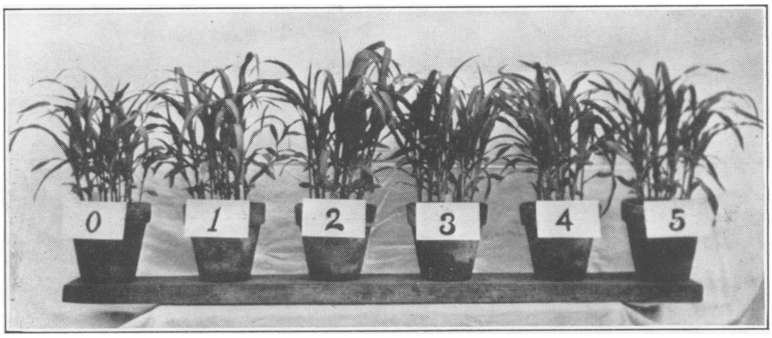

FIG. 2. X-rays and the growth of corn. Control at the left. Time of treatment in minutes indicated on the pots. For other conditions see text.

In table II are presented data on the moisture content of the roots and stems. While the differences are small, they affect roots and tops alike.

TABLE II

WATER CONTENT OF X-RAYED CORN PLANTS

TREATMENT	ROOTS		TOPS	
	DRY WEIGHT	WATER	DRY WEIGHT	WATER
	per cent.	*per cent.*	*per cent.*	*per cent.*
Control	9.34	90.66	8.58	91.42
1 min.	8.09	91.91	8.32	91.57
2 min.	8.75	91.25	8.03	91.97
3 min.	6.78	93.22	8.25	91.75
4 min.	7.92	92.08	7.87	92.13
5 min.	9.14	90.86	9.06	90.94

With light doses, the dry weight percentage decreases and the water content increases. Even these small differences are large enough so that the practiced eye and touch can detect the greater succulence of the plants from seeds treated for 1–3 minutes.

Oats

Only one experiment has been performed with oats. The seeds were from a laboratory sample without name. The increased growth of treated seeds was irregular, as in the case of corn, but plainly visible in all of the treated material. Figure 3 shows the results with plants from seeds

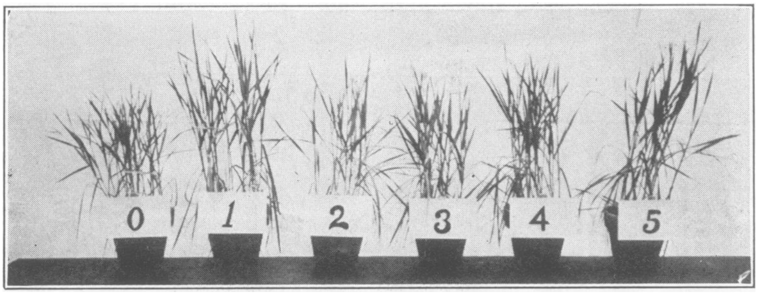

Fig. 3. X-rays and the growth of oats. Control at the left. Time of treatment in minutes indicated on pots. For other conditions see text.

rayed through a 1-mm. aluminum screen at 30 cm. for the periods of time marked on the pots. A defective contact in the machine is believed to have been responsible for the irregular behavior at 2, 3, and 4 minutes, but even these showed increased growth in height and thicker culms than the controls.

Sunflower

The sunflowers were treated after the x-ray machine had been repaired. In figure 4 the controls and treated plants show an excellent curve of height growth. In the photograph the 2-minute and 4-minute plants were omitted. They were perfectly intermediate between 1 and 3 minutes, and 3 and 5 minutes respectively. The 10-minute plants were rayed without the screen. These unscreened plants show the symptoms of burning described by Johnson (**2**). The leaves are asymmetrical, distorted, pocked as if they had mosaic, and the plants are greatly stunted. The screened plants show none of these ill effects; leaves are normal in every way, and growth more rapid. The group of plants rayed 3 minutes blossomed first, indicating a slight shortening of life history by the treatment.

Some attention has been given to the carbohydrate metabolism and respiration of treated seeds. Under the methods we are using, a slightly more rapid liberation of sugar is detectable from the reserves of corn, and a slightly more rapid respiration of rayed seedlings. The increases are not very striking, and we feel that the data are too meager to be published at present. It seems hardly possible that the increased rate of emergence of seedlings, increased rate of growth, etc., could take place without some increase in respiration rate. This may be controlled in part by the concentration of sugar in the protoplasmic environment. The first tests on diastatic activity, however, showed distinct depression of the enzyme by x-ray treatment. Much more extensive tests must be made on sugar concentration, respiration, and enzyme activity with material more favorable than corn for this purpose.

Conclusion

From the results obtained in these preliminary experiments it is concluded that if the x-rays are properly filtered to decrease the intensity of the beam, or to decrease the proportion of the longer radiations, and if the quantity of energy used is adjusted to the specific requirements of the

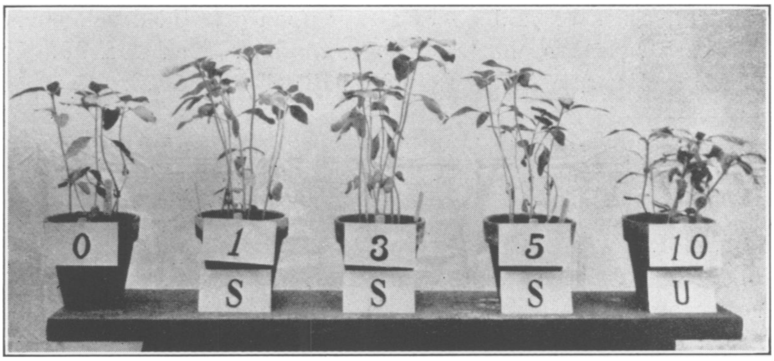

FIG. 4. X-rays and growth of sunflowers. Control at left. Time of treatment in minutes indicated on pots. Plants at right unscreened. For other conditions see text.

plants by control of the duration of radiation, and of the voltage and amperage used, plants can be stimulated to show increased growth rates.

Summary

1. A few preliminary experiments are described which indicate that under appropriate conditions of treatment, x-rays produce stimulative effects upon plant growth. Wheat, corn, oats, and sunflower seedlings have been used.

2. The seeds were treated in an early stage of germination after soaking for 24 hours in a closed moist chamber on a substrate of cellucotton saturated with water. The seeds are not submerged during soaking, but are wet on one side, and in contact with air.

3. The conditions which we believe necessary for such stimulative action are: the use of metallic screens, high voltage and low amperage, and brief exposures. The total dosage for stimulation does not much exceed 100 r-units. Even with the 1-mm. aluminum screen sunflowers given 150–200 r-units were overtreated. Optimum growth occurred with about 115 r-units (3 minutes).

4. There is some evidence of increased sugar content and increased respiration of treated seedlings.

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LITERATURE CITED

1. GOODSPEED, T. H. The effects of x-rays and radium on species of the genus *Nicotiana.* Jour. Hered. **20**: 243–259. 1929. See also Science **67**: 46. 1928, and Natl. Acad. Sci. Proc. **14**: 66–69. 1928.
2. JOHNSON, EDNA L. Effects of x-rays upon growth, development, and oxidizing enzymes of *Helianthus annuus.* Bot. Gaz. **82**: 373–402. 1926. See also Amer. Jour. Bot. **15**: 65–76. 1928.
3. ————. Effect of x-irradiation on growth and reproduction of tomato. Plant Physiol. **6**: 685–694. 1931.
4. ————. Tuberization of potatoes increased by x-rays. Science **68**: 231. 1928.
5. MCKAY, J. W., and GOODSPEED, T. H. The effects of x-radiation on cotton. Science **71**: 644. 1930.
6. PATTERSON, J. T., and MULLER, H. J. Are "progressive" mutations produced by x-rays? Genetics **15**: 495–577. 1930.
7. STADLER, J. L. Genetic effects of x-rays in maize. Natl. Acad. Sci. Proc. **14**: 69–75. 1928.
8. ————. Mutations in barley induced by x-rays and radium. Science **68**: 186–187. 1928.